DENTAL EDUCATIONAL COUNCIL OF AMERICA

Memorandum on the Question Whether Dentistry Should be Developed "Under" Medical Education or, instead, with the Generous Coöperation of Medical Education

ALBERT L. MIDGLEY, D.M.D., Sc.D., F.A.C.D., *Secretary*

Providence, Rhode Island

The Dental Educational Council, at the annual meeting in Chicago last April, adopted the following minute relative to the *Final Report of the Commission on Medical Education*:[1]

"The Dental Educational Council of America has given special attention to the comment on dentistry on pages 216–217 of the *Final Report of the Commission on Medical Education* (December 1932). Included in the Commission's comment is the expressed opinion that 'dentistry should be developed under medical education.' No reasons for this intended subordination of dentistry are given excepting the general opinions that (a) 'it would seem logical;' (b) 'would be consistent with university principles which no longer recognize the artificial separation of fields of knowledge;' and (c) 'would go far toward establishing a highly desirable and valuable correlation of the professional training of these two closely related fields of practice' (medicine and dentistry).

"These suggestions for the subjugation of the dental profession are neither impressive nor persuasive. To the Dental Educational Council, cognizant of the related facts in the history of both medicine and dentistry, the proposed development of dentistry *'under'* medical education, in the United States, (a) would seem to be illogical; (b) would be inconsistent with university principles which clearly recognize the public necessity for the self-determination of, and for appropriate differences in education for, the various professions; and, (c) instead of correlating the professional training in medicine and dentistry, would degrade one profession for the exaltation of the other, and also demoralize the service of the practitioners thus dishonored.

[1] This minute was published on pages 2 and 3 of the cover of the issue of the *Journal of Dental Research* for August, 1933.—[*Ed.*]

"There should be intimate *coördination* between the *principles* and procedures of education for medical practice and for dental practice. Although remaining *independent*, these two forms of health-service education should be made more closely *interdependent* for the betterment of each. The *Council and the dental schools have long promoted this mutual helpfulness.* The strengthening of this constructive educational development, in the public interest, appeals to us as a far more important *interprofessional objective* than the relegation of one profession to a position of *enforced inferiority*. For the attainment of this worthier purpose, we pledge the cordial interest and effective coöperation of the faculties of the dental schools in the United States."

The Secretary was instructed to include the members of the Commission on Medical Education in the distribution of copies of this minute. It gives us great pleasure to present the following responsive comment by Dr. Willard C. Rappleye, Dean of the School of Medicine and Acting Dean of the School of Dentistry, of Columbia University, and Director of the Study described in the *Final Report of the Commission on Medical Education:*[2]

I. LETTER FROM DEAN RAPPLEYE TO THE SECRETARY OF THE DENTAL EDUCATIONAL COUNCIL

July 25, 1933

Referring to the minute adopted by the Dental Educational Council of America concerning the *Final Report of the Commission on Medical Education*, may I say that the only conclusion regarding dentistry on which the Commission expressed itself appears on page 248 of the *Final Report* and reads as follows:

"Education in medicine and dentistry has a common source in the general and medical sciences and should be conducted in a coördinated program under the university, if each division is to meet fully its obligations in the training of professional personnel."

II. LETTER FROM THE SECRETARY OF THE DENTAL EDUCATIONAL COUNCIL TO DEAN RAPPLEYE

August 1, 1933

Your favor of the 25th of July is appreciated. It is a great pleasure to learn that the "only conclusion regarding dentistry on which the

[2] The reader will note that this correspondence is published with the consent of Dr. Rappleye, as indicated in Section III.

Commission expressed itself" in the *Final Report* is the one on page 248 you quote, and which appears among the conclusions at the end of the chapter entitled "The medical course," although it does not appear in the "Summary" at the end of the Report. The statement with which the Council has disagreed appears under the sectional heading, "Dentistry," and was therefore regarded as the Commission's direct, specific, and mature judgment in this relation.

May I report to the Council that in the conclusion on page 248, which you quote as the position of the Commission on "education in medicine and dentistry," the phrase, "conducted in a coördinated program," *does not include* the idea, expressed by the Commission on page 217, that "dentistry should be developed *under* medical education?" I hope you will permit me to make this simple and direct statement, for it would remove the basis for widespread objection to the Commission's subjugation-idea as expressed on page 217, and would enable us to clarify the situation completely to the satisfaction of all concerned.

May I have your permission also to publish, as a unit, your letter of the 25th of July to me, this reply, and your further response? Your permission to do this would enable us to eliminate all occasion for further attention to this matter, and thus to speed the growing accord among the leaders of medical education and dental education in coördinated plans for the promotion of each.

III. LETTER FROM DEAN RAPPLEYE TO THE SECRETARY OF THE DENTAL EDUCATIONAL COUNCIL

August 5, 1933

In reply to your letter of August 1st, we shall be glad to have you call the attention of the Dental Educational Council to the conclusion expressed by the Commission on Medical Education regarding dental education, which appears on page 248 of its *Final Report* and which is consistent with the intent and spirit of the earlier text. This conclusion and the preceding discussion endeavor to emphasize the importance of a closer correlation of the community, research, and educational programs of the two professions. The idea of "subjugation" of the dental profession or of dental education had never occurred to the Commission. In our meetings, the most cordial and friendly attitude

characterized every reference to the problems and contributions of the dental profession and of dental education.

You are at liberty to publish my letter of July 25th, your letter of August 1st, and this reply, if you think that such publication will help in the interpretation of the *Final Report* of the Commission.

IV. LETTER FROM THE SECRETARY OF THE DENTAL EDUCATIONAL COUNCIL TO DEAN RAPPLEYE

September 15, 1933

Your letter of the 5th of August was received with great satisfaction. We are happy to have your assurance that "the idea of 'subjugation' of the dental profession or of dental education had never occurred to the Commission," and, therefore, that the suggestion on page 217 of the *Final Report*, that "dentistry should be developed *under* medical education," to which we took exception, is not one of the Commission's conclusions.

Your response will correct the erroneous impressions created by the said comment on page 217, and we thank you for giving your consent to its publication. The circulation of your comment among dentists will also facilitate the development of the "*intimate coördination* between the principles and procedures of education for medical practice and for dental practice" which the Dental Educational Council declared in its minute to be one of its objectives, and which we are confident you and other leaders in medical education wish to promote.

1108 Union Trust Building.

DENTAL EDUCATIONAL COUNCIL OF AMERICA

MEMORANDUM EXPRESSING A PROTEST AGAINST MISSTATEMENTS IN THE FOOTNOTE ON PP. 112–113 OF THE FINAL REPORT OF THE COMMITTEE ON THE COSTS OF MEDICAL CARE

ALBERT L. MIDGLEY, D.M.D., Sc.D., F.A.C.D., *Secretary*

Providence, Rhode Island

The Dental Educational Council of America has noted the contents of the *Final Report of the Committee on the Costs of Medical Care*. The Council respects all statements of fact and opinion therein contained. The following footnote appears on pp. 112–13:

"*Additional Statement by Committee Members.*—We believe that the following additional recommendation should be made by the Committee:

"c. We commend the growing tendency in the practice of dentistry toward a division of labor in which a dentist who is also a physician assumes larger responsibilities for the diagnosis and treatment of conditions arising from or related to the teeth, while much of the routine performed by the dentist in the past is delegated to dental hygienists and other technicians working under his direction.—*Morris L. Cooke, Haven Emerson, Mrs. Walter McNab Miller, Alfred Owre, William J. Schieffelin, John Sundwall, C.-E. A. Winslow.*"

The foregoing footnote appears in a report that was prepared by an American Committee under American auspices, to describe conditions in the United States, and was intended chiefly for use by the "American people" (p. 71). Where conditions elsewhere than in America are indicated, in the *Report*, the exceptions appear to be carefully noted as such. Thus, the data on "dental care," as outlined on p. 11, obviously relate to conditions in the United States, *for no other nation is mentioned*, whereas a system for the provision of hospital service in England is mentioned as such (p. 69). The said footnote is contained in Chapter V, entitled "The *recommendations* of the Committee," where it is stated (p. 104) that "the Com-

mittee approaches its task of formulating *recommendations* . . . . . [on] the problem of providing satisfactory medical service *to the people of the United States*," etc. The footnote under discussion is included in the section on "*Recommendation 1*" (pp. 109–118), the discussion of which is obviously related solely to American conditions and objectives, excepting the concluding lines on page 118 pertaining to a statement by the British Medical Association. These conditions of presentation in the *Report* show clearly that the footnote on pages 112–13 was intended to state facts relating to the current practice of dentistry among the people of the United States.

The quoted footnote, which purports to be a statement of fact and not an expression of opinion, misleads the uninformed reader into believing that, among "the American people,"

(1) in the practice of dentistry
(2) there is a growing tendency
(3) toward a division of labor, in which
(4) dentists who are also physicians assume larger responsibilities
(5) for the diagnosis and treatment of conditions arising from or related to the teeth, while
(6) much of the routine performed by the dentist in the past (in the practice of dentistry under the indicated condition, i.e., *within the mouth of the patient*) is being
(7) delegated to dental hygienists and other technicians
(8) working under his (dentist-physician's) direction.

This is the same as saying that, in the practice of dentistry among the American people, there is a growing tendency to turn over to dental hygienists and to other technicians much of the actual work of dental practice in the mouths of patients, this dental practice by dental hygienists and other technicians being done, in this growing tendency, under the direction of dentists who are also physicians. We call special attention, in this connection, to the following well-known facts, among many that might be cited:

(1) The dental laws of the states and provinces in the United States and Canada (where they permit practice by dental hygienists) prohibit dental hygienists from performing in the mouth any operations *other than cleansing the teeth;* and no "other technicians" may lawfully do,

in the mouth of a patient, in any state or province, any of "the routine performed by the dentist in the past."

(2) Nowhere in either country is there any tendency, or any growing tendency, to approve or to promote the illegal dental practice *commended* in the said footnote, or to have the statutes amended to make such practice lawful.

(3) In Canada and the United States the number of "dentists who are also physicians"—that is to say, the number of persons licensed to practice dentistry who are or have been licensed also to practice medicine—is smaller now than it was, say, 30, 20, 10 or 5 years ago. These outstanding facts—all well known to dentists—completely invalidate the allegations in the footnote on pages 112–13 of the said *Report*.

The group of seven members of the Committee on the Costs of Medical Care who endorsed the said footnote contains one dentist. About fifteen years ago the same dentist advocated the transformation of dental practice into what the footnote alleges to be a present growing tendency. His preference soon came to be known descriptively as the "master-servant plan." Shortly after the beginning, in 1921, of the Carnegie Foundation's study of dental education, the director of that study, after careful inquiry in all parts of America, and in harmony with nearly unanimous dental and medical sentiment, rejected the "master-servant plan" as undesirable and impracticable. Until about 1930, the "master-servant plan" remained a dead issue, but in that year it was formally brought to life again by the same dentist in his annual report to the President of the University containing the Dental School of which the said dentist was then the Dean. His recommendation was again promptly rejected overwhelmingly by the dental profession, and received no endorsement from organized medicine, any university, or the Dental School of which the said dentist was then the Dean. Nevertheless, this same dentist, despite his knowledge of the realities of the situation, evidently failed to explain to his six co-signers of the footnote that the alleged "growing tendency" was in fact neither a "tendency" nor anything that was "growing."

We condemn both the perversion of truth to which those who signed the footnote on pages 112–13 have subscribed, and the implication in that footnote that the stated untruth might be useful propaganda

against the integrity of the dental profession. Various correspondents and journals have publicly referred to the misrepresentation in that footnote. Indifference to the obligation publicly to correct the published misstatement about current dental practice among "the American people" cannot be commended. We now formally bring to the attention of those who signed the said footnote an article on pages 81–98 of the issue of the *Journal of Dental Research* for February 1933, in which these misrepresentations are exposed in detail. In that article it is stated that as early as December 13, 1932, all who signed the footnote on pages 112–13 of the *Report* were invited to defend or correct, in that *Journal*, the misstatements in the footnote. In response to our inquiry, we have been informed that neither the group of signers, nor any one of them, has presented to that *Journal* any statement correcting the misrepresentation in the footnote on pages 112–13. We have been unable to learn that any other journal has received a statement, from any of the seven signers, intended to correct any public criticism of the misrepresentation, or to defend the misstatements, in the footnote. The Dental Educational Council of America would be happy to receive corrective statements from the seven who endorsed the footnote, and to whom copies of this memorandum will be sent. Their replies would be entered on the minutes of the Council, and published in dental journals.

On page 97 of the issue of the *Journal of Dental Research* mentioned above, we note this among the summary of general conclusions: "The Committee [on the Costs of Medical Care] as a whole has no power to correct the untruths in the footnote on pp. 112–13. This power resides only in the group of seven sponsors of that footnote." We understand that the correctness of this conclusion has not been denied.

Addressed individually (July 24, 1933) to: Dr. Morris L. Cooke, Germantown Hospital, Philadelphia, Pa. Prof. Haven Emerson, 630 West 168th St., New York City. Mrs. Walter McNab Miller, American Child Health Association, 450 Seventh Ave., New York City. Dr. Alfred Owre, 460 Riverside Drive, New York City. Dr. William J. Schieffelin, 25 East 26th St., New York City. Prof. John A. Sundwall, University of Michigan, Ann Arbor, Michigan. Prof. C.-E. A. Winslow, Medical School, Yale University, New Haven, Conn. Copies were widely distributed, a few days later, to journals, faculties, organizations, etc.

1108 Union Trust Building

OBSERVATIONS OF THE BEHAVIOR OF MOLTEN METAL DURING CASTING

A Correction of Statements by R. E. Myers[1]

WILMER SOUDER, Ph.D.

Bureau of Standards, Washington, D. C.

I. QUOTATION FROM A PAPER BY DR. MYERS[1]

On page 1010 of the *Journal of the American Dental Association* for June, 1933, there appeared an article by Dr. Wilmer Souder of the Bureau of Standards, entitled "Time required to cast dental restorations from molten alloy," in which he described the method of casting metal into molds provided with fused-quartz windows. The impression is left that the idea was original with Dr. Souder inasmuch as he gave no credit to anyone for having employed quartz windows to study behavior of molten metal during casting. As early as July, 1930, I demonstrated the method of substituting a fused-quartz plate for one wall of an investment mold before a group of the faculty at the School of Dentistry, University of Louisville. I also gave demonstrations of quartz-window casting before the Blue Grass Dental Society at Lexington, Ky., in December, 1930, and before the Odontological Society of Western Pennsylvania at Pittsburgh, in November, 1931. At the last-mentioned dental meeting, Dr. Souder also appeared on the program. I am not certain that the principle had not been used before I employed it in July, 1930, but I do know that it was then immediately adopted by a great number of men, and that it became widely known as an experimental procedure long before Dr. Souder read his paper at the meeting of the American Dental Association at Buffalo in September, 1932.

II. PRESENT AUTHOR'S COMMENT

No mention of the use of fused quartz was made in the summary of the article to which Dr. Myers referred. In it I attempted to point

[1] *Journal of Dental Research:* 1933, xiii, p. 278; August.

out the five items I regarded as new or worthy of special attention. I am unable to locate any statement in the article which can justify an inference that I regarded this phase of the experiment as new or original.

Research Paper No. 32 of the Bureau of Standards (Dec. 1928, p. 908) refers to Price's classic experiment in which, more than twenty years ago, fused quartz was used in contact with molten gold. On Jan. 11, 1910, Price reported several experiments in which he cast gold against fused quartz. (Reprint from *Items of Interest;* not dated.) These experiments, while not primarily visual, furnished the idea and assurance of its success.

R. L. Coleman, for a number of years Research Associate at the Bureau of Standards, used fused-quartz tubes in attempting to produce rods of gold-alloy by attaching one end of the tube to a vacuum line and immersing the other end in the molten alloy. We watched the gold advance up the tube. These experiments, which I assisted in planning, were made at the Bureau of Standards about the year 1924.

The movie camera, which for years has been used as a means for the study of rapidly changing phenomena, is not questioned. However, I can state that I used the movie camera about 1927 to photograph the behavior of metal passing through a sprue made of dental investment.

All the above dates are prior to July 1930, the date on which Dr. Myers appears to base the claim for priority.

Fused-quartz plates have been used as windows for high-temperature furnaces, for interferometers used in furnaces, and other high-temperature equipment at the Bureau of Standards and in other laboratories, for so many years and on so many occasions that the thought of claiming credit for the idea never entered my mind.

STUDIES ON TRICHOMONAS BUCCALIS[1]

L. H. BEATMAN, M.S.

Department of Zoology, University of Pennsylvania, Philadelphia, Pa.

Although many studies have been made of the protozoan flagellate of the human mouth, *Trichomonas buccalis* (*fig. 1*), its pathogenicity and occurrence in normal mouths has not been definitely determined. Previous investigators either did not look for, or were unable to find, flagellates in mouths showing no diseased condition. Most authors have expressed an opinion that *T. buccalis* is not pathogenic, but the evidence has not been conclusive. Hogue (7) found that of 50 cases 9 (18 percent) were positive for *Trichomonas*, but stated that in every instance there was a history of pyorrhea alveolaris, acute gingivitis, or abscessed teeth. Hinshaw (5), in a study of 300 cases, recorded 53 (17.7 percent) positive, which were found only in cases of advanced or active pyorrhea. In a study of 186 cases in San Quentin prison, Hinshaw (4) found that 30.6 percent harbored flagellates, but all presented diseased conditions of the mouth. Drew and Griffen (2) noted that 10 percent of their patients were infested with *Trichomonas*, most of the mouths showing symptoms of advanced pyorrhea. Jepps (8), examining 16 cases of diseased mouths of Indians and Chinese in the Federated Malay States, observed that 32 percent harbored this protozoan. Nearly all the above values on incidence were casual observations in investigations of oral abnormalities.

The present study of *T. buccalis* represents an attempt (a) to determine its incidence in a fairly large number of cases; (b) to study the relation of its oral incidence to the condition of the human mouth, to obtain data on its pathogenicity; (c) to note the presence of trichomonads in saliva, as bearing on possible transmission through droplet infection; and (d) to make preliminary studies on its viability in tap

[1] A preliminary report was included in the proceedings of the ninth general meeting (1931) of the International Association for Dental Research: *Journal of Dental Research*, 1931, xi, p. 486.

water and culture media at room and incubator temperatures. Material was obtained from the mouths of 350 adults, most of whom (222) were gravid women—patients at the Antenatal Clinic of the Jefferson Medical College Hospital; the rest were patients at the dental clinics of the University of Pennsylvania and of Jefferson Hospital. Permission to conduct these studies was obtained through the courtesy of Dr. Leopold Goldstein, Jefferson Hospital Antenatal Clinic; of Dr. Nordström, Jefferson Dental Clinic; and of Drs. Appleton and Cook, Dental School, University of Pennsylvania. Special thanks are due to Dr. P. Brooke Bland, Supervisor of the Jefferson Hospital Antenatal Clinic, for his kindness in permitting this survey to be made. I also

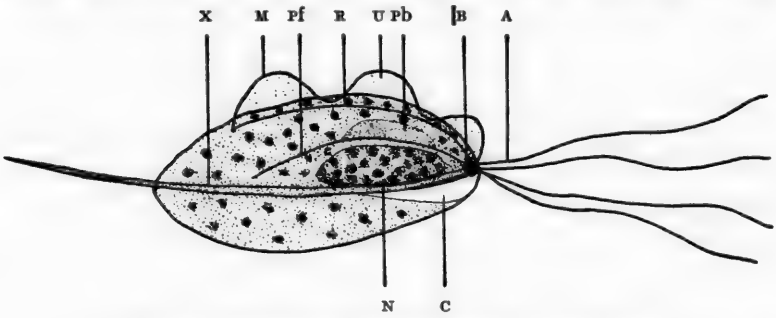

FIG. 1. DIAGRAMMATIC REPRESENTATION OF T. BUCCALIS

A, anterior group of four flagella. *B*, blepharoplast. *C*, cytostome, or mouth. *M*, marginal filament, or posterior flagellum. *N*, nucleus. *Pb*, parabasal body. *Pf*, parabasal fibril. *R*, chromatic basal rod. *U*, undulating membrane. *X*, axostyle. (From drawing by D. H. Wenrich.)

wish to express my deep appreciation to Dr. David H. Wenrich, of the Department of Zoology, for his guidance and assistance.

Culture methods. *T. buccalis* has been cultivated, with various degrees of success, by many investigators since its first successful culture, in 1915, by Lynch (10). Many different albuminous ingredients, such as blood sera, egg albumen, etc., in various salt solutions, have since been used by Ohira and Noguchi (11), Lynch (10), Hogue (7), Hinshaw (6), Deschiens and Kipchidzé (1), and Kipchidzé (9). The present study included a comparative test of the simpler media employed by these authors; additional media were used successfully. Several strains of *T. buccalis* were inoculated into forty-six different media, and the rate and extent of growth in these

were noted. This was done by counting the number of flagellates per microscopic field (for 10 fields, with No. 10 ocular and 16 mm. objective), and comparing the averages obtained each day for each culture medium sustaining the same strain. The media giving the most satisfactory results were modified Hogue's, peptone-liver-extract, rice-starch-Ringer-serum-slant, and dilute Ringer-Loeffler. These preparations, which were used in routine culture work, can readily be made from materials easily available. Their ingredients and preparation, as followed in this study, are given below.

Medium 1: Modified Hogue medium.—(a) Sheep blood-serum diluted three times its volume in distilled water. (b) Ringer solution (grams): NaCl, 8.0; $NaHCO_3$, 0.2; $CaCl_2$, 0.2; KCl, 0.2; dist. water, 1000. (c) Solidified-agar slant. The procedure for the slants is simplified, if commercially prepared nutrient-agar is used. This is dissolved in distilled water in proportions as directed, tubed, autoclaved at 15-lb. pressure for 30 minutes, then allowed to cool and harden in a tilted position. Equal portions of (a) and (b) are mixed and dispensed in test-tubes. These are autoclaved at 15-lb. pressure for 15 minutes. Before inoculation, the sterile mixture is poured over (c). Although somewhat more troublesome and expensive to prepare than many of the other media, this one gave the best results in the routine work.

Medium 2: Peptone-liver-extract medium.—(a) Liver extract (Lilly), 1 gm. (b) Peptone, 1 gm. (c) Ringer solution, 400 cc. These ingredients are thoroughly mixed (solution warmed to 50–60°C.), filtered, tubed, and autoclaved for 15 minutes at 15-lb. pressure. This medium, although very simple, gave consistently good results.

Medium 3: Rice-starch-Ringer-serum-slant medium.—(a) Coagulated sheep blood-serum (3–5 cc.) is dispensed in test-tubes, placed in a reclining position in an autoclave, and sterilized for 15 minutes at 15-lb. pressure. (b) Ringer solution. (c) Rice starch. Ringer solution is poured over (a). The tubes are again autoclaved. A small amount of rice starch is added to the supernatant fluid before inoculation. In this medium, growths of trichomonads were maintained for a long period without subculturing.

Medium 4: Ringer-Loeffler Medium No. 1.—(a) Loeffler dehydrated serum. (b) Modified Ringer solution (grams): NaCl, 6.0; $CaCl_2$, 0.1; KCl, 0.1; $NaHCO_3$, 0.1; dist. water, 1000. Ringer's is autoclaved at 15-lb. pressure for 15 minutes. Before use, the dehydrated serum (0.25 gm.) is dissolved in 100 cc. of warm Ringer solution.

Medium 5: Ringer-Loeffler Medium No. 2.—(a) Loeffler dehydrated serum, 0.5 gm. (b) Ringer solution, 100 cc. Loeffler's is dissolved in this more concentrated Ringer solution in the same manner as above. This

medium, as well as Medium 4, gave profuse growths. It cannot be stored indefinitely; but, because of its simplicity, can be prepared quickly and easily.

For ordinary purposes, the supernatant liquids in these media can be dispensed in 10 cc. amounts. In using media 1, 2, and 3, it is advisable to keep the autoclaved culture-tubes in an incubator (36°C.) several days before inoculating them. This appears to lessen the shock that cold culture-media give to endozoic protozoa; it also tests the sterility of the medium. Drops of cultured material for microscopic examination or subculturing are best obtained with a sterile capillary pipette. It is advantageous to draw off material from the bottom of the tube, among the débris, where the trichomonads collect in greater numbers. To maintain strains in a single test-tube without subculturing, a paraffin-oil layer was used. In this way strains were kept alive for nearly four weeks without transplanting. However, Hogue (7) maintained *Trichomonas* for 30 days; Hinshaw (6), for seven weeks in the same tubes. Addition of saliva to culture tubes to give better growths, as suggested by Hogue, was not found to be advantageous.

The superiority of the culture method of diagnosis over the smear examination was indicated in the following manner. In an attempt to study material fresh from the mouth, smears were placed directly on slides and examined. Of 17 such smears, 4 were positive. On culturing these 17, 8 contained trichomonads. Hegner and Becker (3) have also demonstrated that culture methods give higher percentages of positives.

Incidence. The total of 350 cases were handled by two different methods, in groups of 250 and 100, respectively. In the *first group* (250), sterile swabs were moistened by the patient's saliva, and rubbed over the lower gums—and into pyorrhea pockets, if present. The swabs were then placed in tubes containing culture media. Saliva from the patient was added to this tube, since experiments by Hogue, as mentioned previously, appeared to indicate that saliva favored a better growth of mouth trichomonads. The tubes were carried to the laboratory, placed in an incubator regulated at 36°C., and examined on the third day. In the *second group* (100) most of the mouths were diseased. Each patient was first asked to pass saliva into a tube of culture medium, kept nearly at body temperature. A swab was applied to each mouth, as for the first group, and placed in another test-tube containing the same medium. It was hoped to obtain in this manner an indication of the presence of flagellates in the saliva of *Trichomonas*-infested mouths.

From a comparison of the results recorded in *table 1*, we note that for the *first group*, 49 (19.6 percent) in 250 mouths gave subsequent trichomonad growths; in the *second group*, 28 (28 percent) were positive. The divergence in these percentages may be due to the inequality in the numbers of cases in the two groups. However, mouth condition probably had a greater influence. In the *first group*, 158

TABLE 1

Relative distribution of Trichomonas buccalis in normal and morbid mouths of white and colored patients[1]

PATIENTS: COLOR	MORBID MOUTHS			NORMAL MOUTHS			TOTAL	
	Cases No.	Positive		Cases No.	Positive		Cases No.	Positive Percent
		No.	Percent		No.	Percent		
I. First group: 250 patients								
White	95	26	29.7	52	4	7.7	147	20.4
Colored	63	14	22.2	40	5	13.7	103	18.4
Total	158	40	25.3	92	9	9.9	250	19.6
II. Second group: 100 patients								
White	60	20	33.3	2	1	50.0	62	33.9
Colored	27	5	18.5	11	2	18.1	38	18.4
Total	87	25	28.7	13	3	23.0	100	28.0
III. Groups I and II combined: 350 patients								
White	155	46	29.7	54	5	9.3	209	24.4
Colored	90	19	21.1	51	7	13.7	141	18.4
Total	245	65	26.5	105	12	11.4	350	22.7

[1] "Morbid mouths" were those exhibiting conditions of pyorrhea alveolaris, gingivitis, decayed teeth, or absence of more than three teeth (this indicating a previous diseased condition). "Normal mouths" were arbitrarily adjudged to be those that displayed healthy gums and sound teeth (even though stained with tartar deposits).

(63.2 percent) of the mouths were pathological, while most of the mouths of the *second group* (85 percent) exhibited abnormalities. Considering only results from morbid mouths, it is noted that in the *first group*, 40 (25.3 percent) of the diseased mouths harbored trichomonads, as compared with 25 (28.7 percent) in the *second group*.

The results are illustrated in *fig. 2*, where it is shown that 65 (26.5 percent) of 245 pathological mouths yielded growths of trichomonads,

as compared with 12 (11.4 percent) of 105 normal mouths that harbored the animal. This would indicate that diseased mouths, as well as unclean mouths to a lesser extent, are more favorable for growth of *T. buccalis* than normal, hygienic mouths. The pathogenicity of *T. buccalis* is still doubtful, since any assumption as to what is a normal mouth is in effect arbitrary, and open to error. However, twelve of these arbitrarily designated normal mouths (eight of them in an unsanitary condition) harbored this flagellate, although other observers have found it only in mouths considered pathological. Whereas 46 (29.7 percent) of 155 white patients having pathological mouths were positive for trichomonads, only 19 (21.1 percent) of 90 colored patients harbored this flagellate. From the fact also that 73.5 percent of the diseased mouths apparently harbored no flagellates, there seems to be

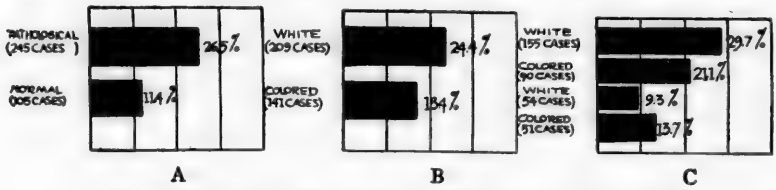

FIG. 2. PERCENTAGE DISTRIBUTION OF T. BUCCALIS (350 CASES)

A, according to condition of mouths. *B*, according to color of patients. *C*, according to condition of mouths and color of patients—*upper pair:* morbid mouths; *lower pair*: normal mouths.

natural resistance to these forms, even though conditions favoring their development predominate. In drawing conclusions from such data, the relative number of cases must be considered. Likewise, it is necessary to assume that all hosts have been exposed to infection, an assumption which doubtless has exceptions.

For the second group, cultures were inoculated with saliva alone. Many of these tubes, after incubation for three days, showed trichomonad growth. Of the 100 cases (28 positive by the swab method), 16 were positive in corresponding saliva-inoculated culture tubes. The presence of *T. buccalis* in saliva of trichomonad-infested mouths suggests possible transmission through droplet infection.

Several experiments were conducted to determine the extent to which different strains of *T. buccalis* were viable in tap water and in culture media at room and incubator temperatures. Equal quanti-

ties of tap water (10 cc.) were inoculated with 10 drops of culture media containing active trichomonads. One set was kept at room temperature (av. 25°C.); the other, incubated at 36°C. Subcultures were made at regular intervals, incubated, and examined in three days. One strain survived submersion in tap water 88 hours at room temperature. The longest time trichomonads survived in incubated tap-water tubes was 64 hours. Survival of trichomonads in tap water was noted by Hinshaw (6) and Hogue (7) for shorter lengths of time. The ability of *T. buccalis* to exist for several days in tap water at room temperature suggests possible transmission through contaminated drinking cups.

To ascertain relative effects of room and incubator temperatures on trichomonad growth in culture media, two strains were each inoculated into three culture media. One set, as before, was kept at room temperature; the other, in an incubator. Subcultures were made at regular intervals, incubated, and examined. The two strains reacted similarly. In one medium (Loeffler-Ringer No. 2), the two strains were maintained for six days when incubated; at room temperature, they remained alive for nine days. In another medium (peptone-liver-extract), trichomonads survived nine days at incubator temperature; at room temperature, twelve days. In the third medium (Medium 3) there was not much discrepancy between the longevity of incubator-cultivated trichomonads and those kept at room temperature. It was noted that the average number of trichomonads per microscopic field (of ten fields counted) was much higher during the first few days in incubated tubes than in those maintained at the cooler environment. The shorter longevity of the former might have been due to the rapid accumulation of waste products, presumably somewhat suppressed in the cooler tubes. The culture of *T. buccalis* at room temperature is important in diagnostic procedure. Should further experimentation indicate conclusively that good growths of trichomonads can be obtained without incubation, routine culture methods to determine the presence of this flagellate may be simplified.

Another interesting observation, which may have some taxonomic significance, was the marked increase in size of the flagellates in media containing rice starch. When the trichomonads were transferred to mixtures lacking rice starch, their size decreased to normal dimensions.

Kipchidzé (9) reported a similar observation in his culture studies of *Trichomonas* in monkeys.

Summary

1. A study was made of the mouths of 350 patients at the maternity and dental clinics of the Jefferson Medical School Hospital and the Dental School of the University of Pennsylvania. In one group (250 cases), 49 (19.6 percent) gave subsequent growths of *T. buccalis* in media incubated at 36°C. Of these, 20.4 percent were white patients; 18.4 percent, colored. Nine normal mouths (8 in an unsanitary condition), of 92 examined, showed trichomonad infestation.

2. A second group of 100 cases was studied to determine the presence of flagellates in saliva of *Trichomonas*-infested mouths. Each patient was asked to pass saliva into a culture tube; then a swab was rubbed over the gums, and placed in a second culture tube. By the swab method, 28 were positive. Of these, 16 showed growth in saliva-inoculated tubes. Presence of trichomonads in saliva suggests the possibility of transmission through droplet infection.

3. Combining the results of the two groups of cases (total, 350) 77 (22.7 percent) harbored *T. buccalis*. Of these, 51 in 209 (24.4 percent) were white patients, and 26 in 141 were colored. Pathological mouths of both races, totaling 65 cases in 245 (26.5 percent), were positive for *T. buccalis*. Material from 12 patients in 105 (11.4 percent) having normal mouths (8 were not kept clean) yielded trichomonad growths.

4. These data indicate that *T. buccalis* can associate with disease-producing organisms, and that unsanitary oral conditions favor its growth.

5. The culture method of diagnosis is superior to the smear method. Of 17 smears taken, only 4 were positive by direct examination, but 8 yielded growths.

6. A comparative study was made of the rate and extent of growth of several different strains in forty-six culture media, many of the latter having been reported as giving good results. Five were found best suited for routine culture work. Modified Hogue medium gave the most consistent and prolific numbers. Increase in size of the trichomonads in rice-starch media was noted.

7. Preliminary experiments on the viability of *T. buccalis*, in tap water at room and incubator temperatures, indicated that flagellates persisted 88 hours at room temperature (average 25°C.) as compared with survival for 64 hours in tap water incubated at 36°C. Transmission through contaminated drinking cups is suggested.

8. The relative longevity of two strains in three different culture media was determined at room and incubator temperatures. In one medium flagellates survived 6 days when incubated, but persisted with fewer numbers at room temperature for 9 days. In another medium the organisms were active for 9 days in a temperature of 36° C., but were maintained for 12 days at room temperature. It was noted that the average number of trichomonads per microscopic field was much higher in incubated media. Incubation might have shortened the longevity of the contained flagellates by favoring more rapid accumulation of toxic waste-products than in the cooler media. More experimentation with room-temperature cultivation should simplify diagnostic procedures for the recognition of *Trichomonas*.

LITERATURE CITED

(1) Deschiens, R., and Kipchidzé, N. 1929 *Trichomonas* de la bouche des singes. *C. R. Soc. Biol., 52:* 518.

(2) Drew, A. H., and Griffen, V. D. 1917 The parasitology of pyorrhea alveolaris. *Jour. Roy. Mic. Soc.* (Part 2), p. 185.

(3) Hegner, R., and Becker, E. R. 1922 The diagnosis of intestinal flagellates by culture methods. *Jour. Paras., 9:* 15.

(4) Hinshaw, H. C. 1926 Correlation of protozoan infection of the human mouth with the extent of certain lesions in pyorrhea alveolaris. *Proc. Soc. Exper. Biol. Med., 21:* 71.

(5) ——— 1927 On the morphology and mitosis of *Trichomonas buccalis* (Goodey) Kofoid. *Univ. Cal. Publ. Zool., 29:* 159.

(6) ——— 1927 Cultivation of *Trichomonas buccalis. Univ. Cal. Publ. Zool., 31:* 31.

(7) Hogue, M. J. 1926 Studies of *Trichomonas buccalis. Am. Jour. Trop. Med., 8:* 85.

(8) Jepps, M. 1923 Protozoa of the human mouth in Malay. *Jour. Paras., 15:* 343.

(9) Kipchidzé, N. 1930 Recherches sur la culture de *Trichomonas elongata. Bull. Soc. Path. Exot., 23:* 216.

(10) Lynch, K. M. 1915 Cultivation of *Trichomonas* and differentiation of the flagellates. *Jour. Am. Med. Assoc., 79:* 1130.

(11) Ohira, T., and Noguchi, H. 1917 The cultivation of the trichomonas of the human mouth (*Tetratrichomonas buccalis*). *Jour. Exper. Med., 25:* 341.

SOLUBILITY OF ENAMEL[1]

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It has been shown by various investigators that enamel dissolves in acid solutions, but the actual H-ion concentration at which decalcification begins has not been agreed upon. McIntosh, James, and Lazarus-Barlow (1) found that solution of enamel begins at pH 4, lower concentrations of H ions having no effect. Rodriguez (2) failed to obtain decalcification at pH 3.8 in ten weeks. Hartzell and Larson (3) did not observe appreciable decalcification at pH 2 even after exposure for four months. All these investigators immersed the whole tooth in the solution, and determined only the original pH value; since both teeth and bone exert marked neutralizing action on acids, it is safe to assume that the acidity of the solutions was greatly diminished during the exposures. Bunting and Palmerlee (4), covering the bulk of teeth with shellac to leave only small areas exposed, obtained decalcification in solutions of pH 2.5, 3.8, and 4.0, in eleven days; and in solutions of pH 4.8 and 5.0 in four and one-half weeks. Friesell and Vogt (5) allowed dilute acid solutions to flow slowly over teeth, and found that lactic acid solutions of pH 4.0–4.1, 4.65–4.8, and 5.0–5.1 produced definite etching of enamel in six weeks. Water, fully carbonated at atmospheric pressure, caused several opaque white spots to appear in three days; but carbonated water, saturated with tricalcium phosphate, did not etch enamel even in eight weeks. McClelland (6), Benedict and Kanthak (7), and Enright, Friesell, and Trescher (8) determined the solubility of enamel in buffered solutions of different pH values. McClelland obtained solution of enamel at all pH values below 6, but no solution in those between pH 6.5 and 11. Benedict and Kanthak found enamel distinctly soluble in acid buffer-solutions, and slightly soluble in distilled water and alkaline buffer-

[1] Proceedings of the eleventh general meeting of the International Association for Dental Research: *Journal of Dental Research*, 1933, xiii, p. 192.

solutions. Addition of either Ca or phosphate ions diminished the solubility. They concluded that were it not for the protective action of these ions, enamel would dissolve in ordinary saliva. Enright, Friesell, and Trescher obtained a distinct action on enamel, as shown by marked whitening effect and loss of polish, at all pH values below 7.4, but at no pH value above 5 if the solutions previously were practically saturated with tricalcium phosphate.

These results published by Enright, Friesell, and Trescher are of great interest; but the conclusion that a pH value of less than 5 is necessary for the production of dental caries seems hardly justified from their experimental results. They used solutions practically saturated with tricalcium phosphate and consequently containing, at the lower pH values, Ca and P concentrations greatly in excess of those occurring in normal saliva. It is probable that, if they had used solutions having Ca and P concentrations comparable to those occurring in saliva, they would have obtained decalcification at pH values greater than 5, since Benedict and Kanthak obtained some enamel solubility in a solution of pH 5.83, and containing 8.23 mg. of Ca and 23.3 mg. of P per 100 cc. However, it is probable that enamel is dissolved by secreted saliva only in exceptional cases, when the salivary pH is extremely low. Thus, Enright, Friesell, and Trescher (8) have calculated, from results obtained by Vogt (9), that the ion product of Ca and phosphate in saliva of both caries-susceptible and caries-free individuals exceeds the solubility product of tricalcium phosphate, showing that saliva is, at least usually, supersaturated by this salt. It is therefore apparent that we must look for the cause of dental caries beyond a simple solution of enamel in saliva itself. Microörganisms undoubtedly play an important rôle through acid production, but no available explanation seems adequately to account for the appearance of caries in certain individuals only. If one assumes a difference in the oral flora of caries-susceptible and caries-free individuals—there is considerable evidence that this is the case—it then becomes necessary to explain this difference. It is possible that variations in the pH, and in the acid-neutralizing action of saliva, may be important factors, caries resulting when these are low, and "immunity" when they are high. Unpublished results obtained by the author, in this regard, are suggestive but inconclusive owing to apparent ex-

ceptions. It is however possible that, if the various cases were followed over a period of sufficient length, the irregularities could be explained.

In an attempt to obtain further information on the origin of caries, it was decided to study effects of the following conditions on solubility of enamel: (a) Effect of magnesium salts, for soluble magnesium salts greatly increase solubility of bone in water (10). (b) Effect of CO_2 in a concentration approximating that of alveolar air. (c) Effect of microörganisms from the mouth of an individual relatively "immune" to caries for a number of years, when a suitable substrate for acid production is present. (d) Effect of the initial pH value of solutions on the rapidity of the decalcification caused by these microörganisms.

Procedure. Enamel, obtained from air-dried teeth by cracking and removing adherent dentine with a dental bur, was finely ground in a glass mortar, and approximately 50 mg. used for about 75 cc. of the respective solutions. No distinction was made between carious and normal teeth, preliminary experiments having shown that the solubility of enamel from carious and from normal teeth was the same in water saturated with air containing 5 percent of CO_2 by volume. The CO_2-air mixtures were prepared by forcing the required amount of CO_2 into a gas holder, followed by air to give an approximately 5 percent concentration of CO_2 by volume. This gas mixture was then allowed to bubble slowly through a train, usually of four solutions, in an incubator at 37°C. Since the gas in the holder was kept over water, the gas would consequently be saturated with water vapor at room temperature and, in passing through the solutions, could cause only very slight evaporation, even in the first solution. Any error resulting from this cause would therefore be negligible. The bubbling gas served not only to keep the solutions saturated with CO_2 at the concentration used, but also to stir them and enable equilibrium to be established rapidly. A similar procedure was used for the air-saturated solutions. In all other experiments, equilibrium was speeded by placing the solutions in a rotatory shaker in an incubator at 37°C.

The solutions in the experiments recorded in *tables 4* and *5* are not "artificial saliva" in the true sense of the term. They contained no protein, but had approximately normal P and bicarbonate concentrations. The Ca concentration was considerably less in most cases than in normal saliva owing to its slight solubility in the solutions used. In preparing the solutions, 0.68 gram of KH_2PO_4, a small amount of $CaCl_2$, and approximately

0.8 gram of $NaHCO_3$ were dissolved in water and the solution made up to 1 liter. From this stock solution the other solutions were prepared by adding a small amount of either $NaHCO_3$ on HCl, to give the desired pH

TABLE 1

Effect of $MgSO_4$ on solubility of finely ground teeth in water, in presence or absence of calcium and phosphate ions

EXP. NO.	DURATION	CONCENTRATIONS OF CALCIUM AND PHOSPHORUS, IN MG. PER 100 CC. OF SOLUTION									
		0.04 *M* $MgSO_4$				0.004 *M* $MgSO_4$		WATER			
		Sol. 1		Sol. 2		Sol. 1		Sol. 1		Sol. 2	
		Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
	hrs.										
1	0	9.4	2.9	0	0	9.4	2.9	9.4	2.9	0	0
	24	10.4	2.7	1.1	0.1?	7.8	2.4	5.3	1.9	0.3	0.3
	48	10.1	2.5	1.8	0.7	7.9	...	5.2	2.1	0.4	0.4
	96	10.9	2.1	1.8	0.7	7.3	2.0	3.3	1.6	0.5	0.5

TABLE 2

Effect of CO_2 on solubility of finely ground teeth in water and in 0.04 M $MgSO_4$

EXP. NO.	DURATION	CONCENTRATIONS OF CALCIUM AND PHOSPHORUS, IN MG. PER 100 CC. OF SOLUTION								REMARKS
		0.04 *M* $MgSO_4$				Water				
		Sol. 1		Sol. 2		Sol. 1		Sol. 2		
		Ca	P	Ca	P	Ca	P	Ca	P	
	hrs.									
1	48	1.1	1.1	2.2	1.2	1.6	0.7	1.4	0.6	Expired air bubbled occasionally through solutions
	96	4.8	1.8	5.7	1.8	2.4	0.8	2.2	0.6	
	192	6.4	2.6	8.3	2.6	2.9	1.4	3.1	1.4	
	268	6.9	2.6	8.7	2.7	3.6	1.6	...	1.7	
2	48	8.2	2.2	7.4	2.1	2.7	1.2	3.0	1.6	5 percent CO_2 bubbled through all solutions
	72	11.7	2.7	10.8	2.6	4.8	1.8	4.4	1.9	
	96	11.7	2.8	11.3	2.8	3.5	1.8	4.1	2.0	
	120	11.7	2.8	11.6	2.8	3.4	1.9	4.1	2.0	
3	24	2.9	1.1	3.7	1.6	2.8	1.1	2.5	1.0	15 percent CO_2 bubbled through all solutions
	72	5.8	2.5	7.0	3.0	4.8	1.4	5.2	1.4	
	96	7.6	3.5	9.0	4.3	6.6	2.9	5.4	2.6	
	148	10.1	4.7	11.6	4.9	6.8	3.1	7.7	3.0	

value. They were then kept in an incubator at 37°C. for several days and any ensuing precipitate filtered off before using.

The saliva in the glucose experiments was obtained from an individual

TABLE 3

Effect of bacterial growth on solubility of enamel in solutions saturated with air or with 5 percent CO_2

EXP. NO.	DURATION	CONCENTRATIONS OF CALCIUM AND PHOSPHORUS, IN MG. PER 100 CC. OF SOLUTION								REMARKS
		Solution 1		Solution 2		Solution 3		Solution 4		
		Ca	P	Ca	P	Ca	P	Ca	P	
	hrs.									
1	24	2.0	...	2.1	...	3.1	...	2.9		5 percent CO_2 bubbled through all solutions. Distilled H_2O used in 1 and 2; 0.004 *M* $MgSO_4$ in 3 and 4
	48	2.1	1.0	2.1	1.1	3.1	1.3	3.1	1.3	
	72	2.0	1.0	2.2	1.1	3.4	1.3	3.2	1.2	
2	24	5.6	...	5.2	...	4.5	...	4.0		Enamel residues from experiment 1 washed, and fresh solutions added using 0.5 percent glucose instead of distilled water; 1 drop of saliva added
	48	6.3	3.1	5.6	3.2	5.3	2.6	6.2	2.8	
	72	8.7	...	9.9	...	9.9	...	10.3		
	96	10.8	4.9	12.4	5.8	13.7	5.9	14.1	6.5	
	120	13.2	6.2	16.7	8.1	21.2	9.8	20.1	9.6	
3	24	2.0	...	1.6	...	1.2	...	1.0		Fresh glucose solution and enamel; 2 drops saliva to each. Air bubbled through solutions 1 and 2; 5 percent CO_2 through solutions 3 and 4. No $MgSO_4$ in any
	48	6.6	...	3.7	...	3.6	...	3.6		
	72	10.8	...	7.3	...	8.4	...	8.4		
	96	20.2	...	14.2	...	13.2	...	14.0		

TABLE 4

Solubility of enamel in artificial saliva saturated with 5 percent CO_2

EXP. NO.	DURATION	CONCENTRATIONS OF CALCIUM AND PHOSPHORUS, IN MG. PER 100 CC. OF SOLUTION											
		Solution 1			Solution 2			Solution 3			Solution 4		
		Ca	P	pH	Ca	P	pH	Ca	P	pH	Ca	P	pH
	hrs.												
1	0	1.0	13.4	7.3	1.0	13.4	7.3	3.1	13.8	6.9	3.1	13.8	6.9
	24	2.0	....	...	1.8	....	...	2.7	....	...	2.1		
	48	2.1	13.6	...	1.9	13.6	...	2.2	14.0	...	2.0	14.0	
	72	1.9	13.6	6.9	1.8	13.6	6.9	2.3	13.8	6.8	2.6	13.8	6.9
2	0	1.2	14.9	7.2	1.2	14.9	7.2	3.4	14.9	6.8	3.4	14.9	6.4
	24	1.5	....	...	1.4	....	...	1.2	....	...	1.3		
	48	1.4	13.3	...	1.4	13.3	...	1.5	13.3	...	1.6	13.3	
	72	1.2	14.1	7.1	1.2	13.3	7.1	1.3	13.4	6.9	1.2	13.4	6.4
3	0	6.6	14.1	5.7	6.6	14.1	5.7	6.6	14.1	4.8	6.6	14.1	4.8
	24	6.6	....	...	6.6	....	...	7.6	....	...	7.5		
	48	7.2	13.7	...	7.3	....	...	7.9	14.8	...	7.7	14.4	
	72	7.3	14.1	5.7	7.2	14.1	5.7	8.0	14.4	5.4	7.7	14.5	5.4

practically immune to dental caries for a number of years. The same amount of saliva was used in all solutions in each experiment. Ordinary well-washed laboratory glass-ware was used. No special precaution was taken to prevent contamination from air-borne organisms, since the purpose of the experiment was not to study the effect of any particular organism, but of the collective action of such organisms as were present in the subject's mouth, or to which he was exposed; and which would naturally

TABLE 5

Solubility of enamel in artificial saliva containing 0.5 percent glucose and inoculated with saliva. (Air was slowly bubbled through all solutions during each experiment)

EXP. NO.	DURATION	CONCENTRATIONS OF CALCIUM AND PHOSPHORUS, IN MG. PER 100 CC. OF SOLUTION											
		Solution 1			Solution 2			Solution 3			Solution 4		
		Ca	P	pH	Ca	P	pH	Ca	P	pH	Ca	P	pH
	hrs.												
1	0	1.0	13.6	7.3	1.0	13.6	7.3	3.1	13.8	6.9	3.1	13.8	6.9
	24	1.0	....	...	1.4	....	...	3.5	....	...	3.2		
	48	2.0	....	...	5.1	....	...	14.0	....	...	8.0		
	72	6.7	17.4	...	9.8	18.3	...	18.6	21.8	...	13.8	19.4	
2	0	1.2	14.9	7.2	1.2	14.9	7.2	3.4	14.9	6.8	3.4	14.9	6.4
	24	2.7	....	...	2.7	....	...	3.7	....	...	4.4		
	48	5.9	....	...	5.1	....	...	10.2	....	...	11.0		
	72	11.8	18.7	4.8	9.0	17.5	5.2	16.1	20.5	4.7	17.1	20.5	4.6
3	0	6.6	14.1	5.7	6.6	14.1	5.7	6.6	14.1	4.8	6.6	14.1	4.8
	24	11.2	15.2	...	10.3	15.2	...	10.0	15.6	...	11.0	15.6	
	48	16.4	16.4	...	14.8	15.8	...	12.9	15.6	...	13.5	15.7	
4	0	3.4	15.2	7.3	3.4	15.2	6.5	3.4	15.2	6.3	3.4	15.2	5.7
	24	2.2	15.2	...	4.1	16.6	...	4.6	16.6	...	5.7	17.5	
	48	4.5	15.5	5.6*	8.2	....	4.6*	10.2	17.2	4.4*	13.7	19.0	4.0*
5	0	3.4	15.2	7.3	3.4	15.2	6.5	3.4	15.2	6.3	3.4	15.2	5.7
	24	1.0	13.9	....	3.2	14.9	...	4.4	15.4	...	10.4	15.9	
	48	1.4	14.0	7.2*	4.3	15.1	4.8*	10.9	17.8	4.2*	21.9	22.3	4.0*

* The pH values were determined colorimetrically.

multiply in his mouth, if given a suitable environment. A small amount of chloroform prevented bacterial growth in all experiments in which glucose was not used.

Method of analysis. Ca was precipitated as oxalate, washed, and titrated with potassium permanganate in the usual manner. P was determined by Fiske and Subbarow's method (11). The pH determinations were made with quinhydrone electrodes unless otherwise mentioned. The electrode vessel previously described (12) was used in determining the pH of solutions

containing CO_2, and the gas mixture was bubbled through the solutions during pH measurements.

Discussion. It will be seen, from the results recorded in *tables 1, 2,* and *3,* that magnesium sulfate increases the solubility in water of finely ground teeth and enamel, in the presence or absence of CO_2. The decrease in Ca and P concentrations, in solutions 3 and 4 (*table 1*), was probably due to decreased acidity from the basic action of the teeth. However, presence of magnesium sulfate diminished the amount thus precipitated out; when sufficient magnesium was present, actual solution of tooth substance took place (solution 1, *table 1*). The mechanism of this solvent action of magnesium salts on bone and teeth is not understood. It may be due to an increase in the solubility-product constant of Ca salts of bone and teeth, or to the formation of a soluble, complex Ca-Mg salt. It is probable that this solvent action of Mg is not an important factor in dental caries, since only small amounts of Mg are present in saliva.

The solvent action noted with CO_2 seems to be due to its acidic properties. Its effect is largely determined by the initial Ca and P concentrations and the pH of the solution. The decrease in Ca in solutions 3 and 4 (experiments 1 and 2, *table 4*), without any appreciable change in pH, may be due (a) to supersaturation in regard to tricalcium phosphate, the excess separating out from contact with the enamel; or (b) to the formation of a more insoluble compound than tricalcium phosphate, the reaction being speeded up by the presence of enamel. However, irrespective of the cause of the decrease, it seems conclusive that no decalcification takes place at any pH above 6.4, if the Ca- and P-concentrations are as high as 3 and 14 mg., respectively, even when the solution is saturated with CO_2 at a tension undoubtedly as great as that of any normal expired air. At a pH of 5.7, slight decalcification takes place even with an initial Ca concentration of 6.6 mg. per 100 cc. of solution.

It is probable that only in exceptional cases are the pH, and also the Ca- and P-concentrations, of freshly secreted saliva sufficiently low to dissolve enamel. However, if acid-forming organisms are present, in conjunction with a suitable substrate for acid formation, decalcification occurs (*tables 3* and *5*). Decrease in the initial pH-values of the solutions, down to a certain point, favors decalcification. This in-

crease in decalcification, with increase in solution-acidity, is especially noticeable in experiments 4 and 5 (*table 5*) and is indicated in *fig. 1*. Initial decrease of Ca, and in one case of P, in the solutions of pH 7.3 containing 3.4 mg. of Ca per 100 cc., might have been due (a) to removal of dissolved CO_2 by aeration, and consequent decrease in solubility of both tricalcium phosphate and calcium carbonate; or (b) to formation of a more insoluble compound than either of these substances. The greater decalcification in solutions of pH 5.7 than in

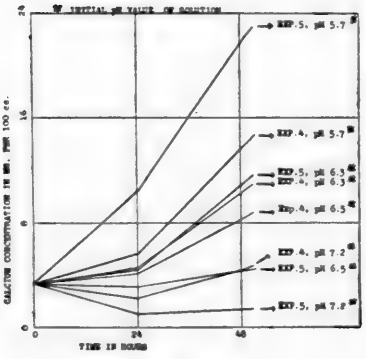

FIG. 1

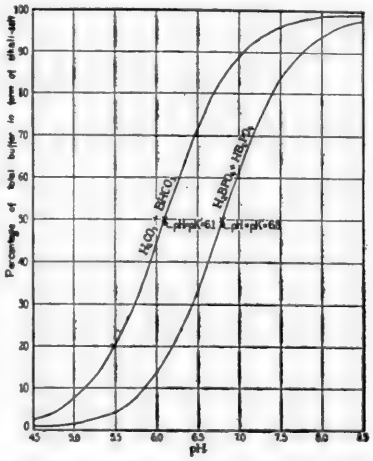

FIG. 2

FIG. 1. Decrease in initial pH value favors decalcification of enamel; illustrative data from records of experiments 4 and 5, *table 5*.

FIG. 2. Curves showing pH of solutions of H_2CO_3 and H_2PO_4, of which varying percentages are combined with alkali to form $BHCO_3$ and $BHPO_4$, respectively.

those of pH 4.8 (experiment 3, *table 5*) appears to have been due to inhibitory action of the former on the growth of the acidogenic organisms. Borgs (13) has recently shown that acidogenic organisms from a carious lesion produced a minimum pH of 4.0 when grown on glucose-broth media; but when transplanted from agar media of pH 7.6 to glucose-broth media of various H-ion concentrations, no growth nor acidity resulted at pH 4.7 although they occurred at higher pH values.

Increase in rate of decalcification with increase in H-ion concentra-

tion of solutions, within limits, is probably due primarily to reduction in acid-neutralizing power of the solutions with decrease in pH value; but it is also possible that slightly acid media favor growth of these acidogenic organisms and consequently increase rate of acid formation. The main buffers of saliva are undoubtedly phosphates and carbonates. Their marked decrease in acid-neutralizing power, with decrease in pH, can be readily seen from *fig. 2*, taken from Peters and Van Slyke's book (14). For example at pH 7.2, approximately 72 percent of the phosphate and 93 percent of the total CO_2 are present as the basic salt; at pH 6 only 13.3 percent of the phosphate and 44 percent of the CO_2 are present as the basic salt. It therefore appears evident that the amount of acid which would induce caries in an individual whose saliva has low acid-neutralizing action—other factors such as salivary flow, etc., remaining constant—would not cause caries in an individual whose saliva has high acid-neutralizing action.

The importance of readily fermentable carbohydrates in the etiology of caries is obvious. It is probable that carious lesions could be produced in any individual if a sufficient amount of glucose could be continually maintained in the mouth, but it is obvious that many other factors besides fermentable carbohydrates are important. The flow of saliva and its pH and acid-neutralizing action, the accessibility of various portions of tooth surface to saliva, as well as conditions favoring food impaction, must be important contributing factors in determining the presence or absence of the lesion.

Summary

(1) Magnesium sulfate increases the solubility in water of finely ground teeth and of enamel, in the presence or absence of carbon dioxide. It is believed however that, owing to the small amount of Mg normally present in saliva, it cannot be considered an important factor in the etiology of dental caries.

(2) Carbon dioxide, even in low concentrations, has definite solvent action on enamel in the absence of Ca ions and phosphate ions in the original solution. This solvent action is probably due to the acidic properties of CO_2 when dissolved in water.

(3) If as much as 3 mg. of Ca and 14 mg. of P are present in 100 cc. of solution, no decalcification is produced by 5 percent of CO_2 at pH

values of 6.4 and above; but slight decalcification is produced at pH 5.7, even with an initial Ca concentration of 6.6 mg. per 100 cc. of solution.

(4) If glucose and acidogenic organisms are present in the solutions, decalcification results: especially readily at an initial pH 5.7; rather slowly at pH 7.3; at intermediate rates at intermediate pH values.

REFERENCES

(1) McIntosh, J., James, W. W., Lazarus-Barlow, P. 1922 *Brit. J. Exper. Path.*, *3*, 138.
(2) Rodriguez, F. E. 1922 *Mil. Dent. J.*, *5*, 199.
(3) Hartzell, T. B., and Larson, W. P. 1924 *J. Am. Dent. Assn.*, *11*, 649.
(4) Bunting, R. W., and Palmerlee, F. 1925 *Ibid.*, *12*, 381.
(5) Friesell, H. E., and Vogt, C. 1926 *Ibid.*, *13*, 748.
(6) McClelland, J. R. 1926 *Dent. Cosmos*, *68*, 127.
(7) Benedict, H. C., and Kanthak, F. F. 1932 *J. Dent. Res.*, *12*, 277.
(8) Enright, J. J., Friesell, H. E., and Trescher, M. O. 1932 *Ibid.*, *12*, 759.
(9) Vogt, C. Quoted by Enright, J. J., Friesell, H. E., and Trescher, M. O. 1932 *Ibid.*, *12*, 759.
(10) Forbes, J. C. 1931 *J. Biol. Chem.*, *93*, 255.
(11) Fiske, C. H., and Subbarow, Y. 1925 *Ibid.*, *66*, 375.
(12) Forbes, J. C. 1932 *J. Dental Res.*, *12*, 749.
(13) Borgs, F. 1930 Dissertation; University of Hamburg, Germany.
(14) Peters, J. P., and Van Slyke, D. D. 1931 Quantitative clinical chemistry, Vol. 1, page 891; Williams & Wilkins Co., Baltimore, Md.

DOES PASTEURIZED MILK CAUSE VARIATION FROM NORMAL IN THE CRYSTAL STRUCTURE OF ENAMEL FORMED DURING THE PERIOD OF USE OF SUCH MILK?[1]

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The value of *raw* milk in the diet, to prevent caries of enamel of teeth developed during the period of feeding such milk, has recently come into prominence owing to observations by Sprawson (1). Data on groups of institutional children, covering years of notation, leads him to believe that *raw* milk confers a notable degree of caries immunity on enamel of teeth developed during a period of feeding of raw milk. In an attempt to explain the probable cause of such immunity, Sprawson states: "I have always thought that the important point is not the percentage of lime salts in the enamel so much as their stability, or the stability of the compounds which they form with the organic matrix." It occurred to the authors that such a condition of stability or lack of stability might be apparent in the optical properties of the crystal structure of enamel of incisors of rats on diets of raw milk, and of milk from the same supply after pasteurization. Since incisors of white rats grow constantly they should reflect variations in diet, provided such variations are optically demonstrable and the diet is ingested over sufficiently long periods to result in complete replacement of the growing tooth tissues.

In a previous article by one of us (2), various phases in the formation of enamel of the rat incisor, as observed under polarized light, were described. It is believed, on the basis of these observations, that (a) the ganoblasts lay down a complete organic matrix free from any crystal deposits; (b) that this matrix is later impregnated initially, in the walls of the organic rods, by crystals of a positive optical-sign which (c) then change through an isotropic state into crystals of a negative optical-sign, (d) the entire organic matrix, both septated tubes and enclosed globules, being finally impregnated by crystals

[1] Proceedings of the eleventh general meeting of the International Association for Dental Research: *Journal of Dental Research*, 1933, xiii, p. 187.

of a negative optical-sign, thus producing the physiological enamel. Since this series of phenomena occurs in a quite uniform fashion in enamel of rats on a normal diet, it was thought that deviations might result from dietary departures from normal. Accordingly we placed three groups of five rats each on the following diets: *Group I* (controls).—"Old Trusty" dog-biscuit; ten drops of cod-liver oil per biscuit; approximately 25 grams of raw carrot per week per rat; water. *Group II* (*raw milk*).—*Raw* milk ad. lib.; carrot as above; water. *Group III* (*pasteurized milk*).—Milk from the supplies used in Group II, but *pasteurized*, ad. lib.; carrots as above; water.

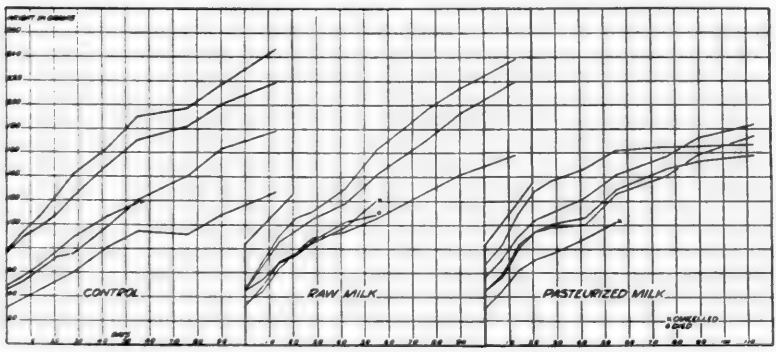

FIG. 1. GRAPHS SHOWING GROWTH OF RATS ON MILK DIETS

The rats were approximately 30 days old when taken; were not all litter-mates; and were kept on the diets for 113 days, with the exceptions noted on the weight graphs (*fig. 1*). The growth of each animal was checked by periodic weighings (*fig. 1*). That pasteurization of the milk affected its nutritive value is apparent from the individual growth-curves. The raw milk diet approached the control diet as a growth promoter. The pasteurized milk caused a levelling off of the growth curve, after a short period of normal gain that was probably due to reserves initially present in the tissues. These growth figures confirm those obtained by Scott and Erf (3) in much larger groups of animals than ours. Since all groups received a quite liberal supply of carrots, and xerophthalmia did not occur in any of the animals, the growth deficiency could not be attributed to any great difference in vitamin-A content of the diets. Vitamin C was probably not a factor,

since the albino rat seems able to overcome lack of this vitamin by its own biological processes. Vitamin D is quite heat-resistant, and we know of no evidence that the pasteurization process interferes with its beneficial effects. Nevertheless, pasteurization seems to reduce the nutrient value of milk for an unknown reason.

To determine whether this dietary deficiency might be apparent in the optical properties of enamel produced during the experimental period, sections of incisors, both upper and lower, were ground to expose sagittal planes for examination in polarized light. Careful study of enamel from all rats in the three groups failed to show any consistent differences in optical properties between enamel developed

TABLE 1

Hemoglobin and red blood-cell conditions in rat groups I, II, and III

DATE	HEMOGLOBIN (GRAMS PER 100 CC.)	R.B.C.	ENAMEL COLOR	GROUP
3–1–32	*11.8*	*6,980,000*	*Dark orange*	*Control*
Same	Too low to read	4,270,000	Colorless	Pasteurized
Same	5.9	5,500,000	Colorless	Raw
4–25–32	*9.8*	*6,100,000*	*Dark orange*	*Control*
Same	4.8	5,100,000	Colorless	Pasteurized
Same	3.7	4,050,000	Colorless	Raw
5–10–32	*10.8*	*6,400,000*	*Dark orange*	*Control*
Same	5.2	5,740,000	Colorless	Pasteurized
Same	2.9	3,100,000	Colorless	Raw

during feeding of pasteurized milk and enamel developed during feeding of some of the same milk in a raw condition. *Microscopically* neither milk-fed group showed consistent variation from the controls.

From the *macroscopic* standpoint both milk-fed groups showed a variation from the controls. This difference lay in the coloration of the labial aspect of incisor enamel, upper and lower. In the controls, this area had a dark-orange appearance, but in both milk-fed groups the color was practically absent. During a discussion with Dr. R. G. Agnew, of Toronto, we learned that he also has noted this lack of enamel coloration in milk-fed rats. While not contending that proof of the cause of this phenomenon is hereby presented, we include in this report a *table* giving periodic findings for hemoglobin and red blood-cells. The data show that loss of enamel pigmentation accompanied low hemoglobin-content and low red blood-cell count; and that

where these conditions were within normal range, as in the control group, the usual enamel coloration was present. As a possible indicator of anemic conditions in experimental rats, a careful comparison between hemoglobin content and red cell-count on one hand, and the shading of the labial aspect of the incisor enamel, might be worth while. It should be noted that even the raw-milk group showed that the milk (winter milk from the ordinary supply of the University Dairy) was not from cows on a diet sufficiently fortified to prevent anemia in rats fed upon their milk. That such fortification of the food of cows favors production of milk capable of preventing anemia in white rats has been demonstrated by the work of Scott and Erf, previously mentioned. However these observers have shown that even such milk, *pasteurized*, is not capable of producing the growth that it induces when fed *raw*.

The control animals showed no anemia. Since the optical properties of the control enamel varied in no way from that of the milk-fed groups, it seems permissible to conclude that anemia, to the degree demonstrated in the experimental rats, causes no variation in the optical properties of enamel laid down during the anemic conditions. At least our technique of observation showed none.

Summary and conclusions. 1. Pasteurization of milk lowers its nutrient value for white rats.

2. White rats, on a milk diet and giving evidence of anemic condition, did not exhibit the dark-orange coloration of the incisor enamel that occurred in the control animals.

3. There were no significant optical differences between enamel of control rats and of milk-fed rats, nor between enamel of animals fed pasteurized milk and of those on raw milk from the same supply. Inability to detect such differences does not necessarily mean that there are none; but the most appropriate available technique has failed to reveal dissimilarities.

We are indebted to Dr. C. A. Doan, of the Department of Medical and Surgical Research, for assistance in the blood determinations.

References to Literature. (1) SPRAWSON, EVELYN. 1932 Preliminary investigation of the influence of raw milk on teeth and lymphoid tissue. *Proceedings of the Royal Society of Medicine, Section of Odontology*, XXV, p. 11. (2) KITCHIN, PAUL C. 1933 Some observations on enamel development as shown in the mandibular incisor of the white rat. *Journal of Dental Research*, XIII, p. 25. (3) SCOTT, ERNEST, AND ERF, L. A. 1932 Effects of cattle feeding and pasteurization on food value of milk. *Annual Proc. of Int. Assn. of Dairy and Milk Inspectors.*

CALCIUM AND PHOSPHORUS STUDIES

VIII. Some Observations on the Incidence of Caries-like Lesions in the Rat[1]

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INTRODUCTION

In spite of numerous investigations aimed to clarify the cause of dental caries, the etiology remains obscure. The theories advanced as to the etiology of dental decay in human beings range from oral sepsis, acidosis, "anticalcifying enzymes" to avitaminosis. Many of these theories have no scientific basis—they are merely speculative—while others are based on evidence derived from animal experimentation. With increasing knowledge in the field of nutrition, especially as pertains to calcium (Ca) and phosphorus (P) metabolism and to vitamins, numerous papers have appeared in support of the thesis that dental caries is caused by a paucity in either Ca or P, or in one or more vitamins. The results of experiments in lower animals have often been applied *in toto* to the problem of dental caries in human beings, and Ca, vitamins A, C and D, and P have had their fashions in dental therapeutics. The P-deficiency theory has been recently revived by several investigators (1, 2, 3). While it is unquestionably true that for ossification to proceed normally an abundance of P as well as Ca in the diet is necessary, and that the P phase in nutrition is frequently neglected, as pointed out previously by Shohl and Bennett (4) and by us (5), yet the experimental evidence that a P deficiency in the diet is the *primary* factor in the causation of dental caries is not conclusive. We have become aware of the invalidity of such an assumption in an analysis of our own data on the incidence of dental caries in over one thousand rats, ranging in age from 30 to 1153 days,

[1] Aided by a grant from the Mead Johnson Company, Evansville, Indiana.

and on diets varying in Ca, P, and vitamin-D contents. If our experiments had been limited to the early ones, the conclusions drawn from them might have been in accord with the P-deficiency theory. But, as the periods of observation were prolonged and the physical as well as chemical composition of the diets was made more varied and the experiments were better controlled, the P-deficiency theory lost validity. Thus, the caries-like lesions that developed in rats on low-P high-Ca diets for about 100 days were also noted in rats on the stock diet but for longer periods. Also, when the coarsely ground corn of the Steenbock rachitogenic and stock diets was replaced by either wheat or casein, caries did not develop, in spite of nearly identical intakes of Ca and P. Apparently, aside from the lime-salt deficiencies in the diets, two other factors were responsible for dental caries in our rats; namely, (a) age and (b) physical make-up of the diet. The following data, in the order of development, indicate the steps leading to these conclusions.

MATERIAL

The animals used for our analyses were obtained from our own breeding and experimental colonies; also from the rat colony of Dr. Charles E. Bills of the Research Laboratories of the Mead Johnson Company.[2] From the latter source only the heads were obtained; and from their examination much of the data on rats older than twelve months was secured. The strain of rat, the breeding care, and the dietary régime in Dr. Bills' laboratory and in ours are nearly identical. The stock and rachitogenic diets, as well as the vitamin D used in both laboratories, are also of similar composition. A total of 1150 heads was examined. In our own animals, the teeth were examined at autopsy, and the presence or absence of macroscopic molar caries[3] was ascertained by gross inspection. The preserved heads of the animals from the Mead Johnson Laboratories were similarly examined soon after their arrival. In most instances the diagnoses of

[2] We are indebted to Dr. C. E. Bills and the Research Staff of Mead Johnson Company for their coöperation.

[3] The word "caries" is used in its broader sense, i.e., caries-like lesions as produced in rats by dietary means.

macroscopic caries were subsequently corroborated histologically.[4] In most of our own rats analyses of blood serum for Ca and inorganic P, and histologic and x-ray examinations of other parts of the skeleton were also made.

EXPERIMENTS AND RESULTS

Most of the data of our early experiments on the incidence of dental caries in rats was obtained from the teeth of animals on either the Steenbock, high-Ca, low-P diet [*2965 (6)], or diets in which the P was optimal or high and the Ca varied between extremely low and just-minimal levels. The former diets were composed chiefly of ground corn and gluten; the latter rations contained either ground wheat or casein and starch. In these groups, gross dental caries was absent in those on the diets optimal or high in P, but prevalent among those on the low-P diet. The findings were the more surprising since the low-Ca diets produced extremely hypoplastic teeth without caries, and the low-P rations produced caries even when vitamin D, which presumably prevents defective calcification of bone, was also added to the diet. The histopathology of the caries-like lesions in the low-P groups was described by Klein and Shelling (7), and the hypoplasia and the absence of dental caries in the low-Ca groups were previously reported by Shelling and Asher (8). The findings mentioned would, at first thought, seem to indicate that a low-P intake predisposed to dental caries, and that a low-Ca diet, though producing dental hypoplasia, was not conducive to dental decay. However, as the observations were extended to rats on other diets, it became apparent that the P intake was not the sole factor in the predisposition to dental decay. Thus, for example, of the 263 rats on the Steenbock-Bills *stock* diet (9) without added vitamin D, for periods ranging from 120 to 1153 days, 141 (60 percent) had dental caries. Similarly, in those receiving this diet with varying amounts of vitamin D for from 30 to 1026 days, caries was found in 108 out of 165 animals. Of the entire group of 401 animals on the stock diet, with or without vitamin D, 249 (62 percent) had caries. Since this diet is apparently adequate for growth, reproduction, and lactation, and contains optimal amounts

[4] We are indebted to Dr. Henry Klein, of the Department of Biochemistry of the School of Hygiene, for the preparation and interpretation of the histologic sections.

of both Ca and P, the high incidence of dental caries among the animals that received this ration could not have been due to paucity in the P intake. The presence of caries was the more surprising in the groups receiving optimal or even high doses of vitamin D in the form of cod-liver oil or viosterol, which ordinarily accentuates lime-salt deposition in the skeleton, and hence might have been expected to prevent dental decay.

Several possibilities were considered as explanations of the predisposition to dental decay in the groups receiving the rachitogenic and the stock rations. The main difference between the diets that gave rise to dental caries and those that did not was in the ingredients which furnished the protein. In the latter diets most of the protein was in the form of either casein or wheat, whereas the chief source of protein in the stock and the Steenbock rachitogenic diets was yellow corn, which constituted 60 to 76 percent of these rations. This fact suggested that the corn might be the cause of dental caries, because either (a) the oils in the corn were toxic to dental tissue, or (b) the corn protein lacks some essential substance necessary for dental ossification, or (c) the physical make-up of the diet, i.e., the coarseness or fineness of the particles, injured the dental enamel by favoring impaction, infection, and decay, as recently suggested by Hoppert, Webber, and Caniff (10). The first possibility seemed unlikely from the fact that in our experiments many of the diets not predisposing to dental caries contained corn oil as a vehicle for diluting viosterol and also from the fact that the siftings from the corn, which presumably retained their oil content, had not produced caries in the animals of Hoppert, Webber, and Caniff. The second possibility also seemed unlikely since, as stated above, caries developed also in more than half the rats on the stock diet, although it contained dry milk and casein, in addition to the corn protein, and insured normal ossification of the skeletal tissue other than the teeth. The last possibility remained to be tested. It would follow that if the corn particles of the diet, and not the P *per se*, were the causative factor in the production of dental injury in rats, caries should be present on corn diets irrespective of the Ca and P intakes, and absent on low-P diets in which the coarse particles of corn were replaced by a softer grain such as wheat.

In the first experiment designed to elucidate this point, we used a low-P optimal-Ca diet composed chiefly of ground whole-wheat, gluten, casein, starch, yeast, fat, and a P-free salt-mixture (Diet 75A, *table 1*). The P content of the diet was about 0.135, and the Ca about 0.660, gram percent. Eight rats, fed this ration from weaning until 217 days of age, failed to show dental caries, in spite of the low-P intake. The experiment was extended to a much larger series and the test diets were modified as follows: (*a*) to the basal diet (75A), Na_2HPO_4 (anhydrous) was added in increments of 0.2 gram percent. In this way a series of six diets was formed in which the P percentage was increased by 0.043 (76A), 0.086 (77A), 0.130 (78A), 0.218 (79A), and 0.327 (80A) gram percent above the basal level of about 0.135 gram percent. (*b*) Two percent viosterol 1-D was added to the above six diets forming series B, and to another set 2 percent of cod-liver oil was added, forming series C. Thus there were eighteen diets in all—six without, and twelve with, vitamin D additions. The respective diets were given to groups of four animals from the time of weaning until they were from 217 to 233 days of age. Those without vitamin D (series A) were given to two groups each, and the viosterol (series B) and cod-liver oil (series C) containing diets to only one group in each of the respective series, the entire series containing 96 rats. At autopsy none of these animals showed evidence of dental decay.

The experiment was further modified by increasing the Ca content of the diet to about 1.260 grams percent, or approximately that contained in the Steenbock diet (# 2965), while the P was left at the former low level of 0.135 gram percent. This diet, without the vitamin D addition, was given to eight rats from weaning until they were 245 days old. The animals fared very poorly on this ration. They gained very little in weight and did not reproduce. But, in spite of the lower P-content as compared with that in the Steenbock rachitogenic diet, and the fact that it was ingested for periods much longer than that required for caries to occur on the Steenbock ration, caries did not develop, although the teeth appeared hypoplastic.

The possibility that the Steenbock-Bills stock diet, although adequate for growth, reproduction, and lactation, may be deficient in the P necessary for the prevention of dental decay was next investigated.

TABLE 1

Composition of diets

1. *Stock diet* (Steenbock-Bills)

	grams
Yellow corn (ground)	76.0
Crude casein	5.0
Linseed oil meal	16.0
Alfalfa meal	2.0
NaCl	0.5
$CaCO_3$	0.5
	100.0

3 parts of diet + 1 part powdered whole milk. Fresh vegetables once a week.

Ca = 0.515, P = 0.450, gram percent

2. *McCollum stock diet*

Wheat (ground)	20.0
Corn (ground)	20.0
Rolled oats (ground)	20.0
Flaxseed meal	10.0
Crude casein	3.5
Whole-milk powder	25.0
$CaCO_3$	0.5
NaCl	1.0
	100.0
Ferric citrate	0.1
$CuSO_4$	0.02

Greens three times weekly.

Ca = 0.475, P = 0.450, gram percent (approximately)

3. *Modified Sherman stock diet* (wheat or corn)

Wheat (ground)	64.3
Powdered whole-milk	33.3
NaCl	2.0
$CaCO_3$	0.4
	100.0
Yellow corn (ground)	63.6
Powdered whole-milk	33.3
NaCl	2.0
$CaCO_3$	0.4
Na_2HPO_4 (anhydrous)	0.7
	100.0

Ca = 0.500, P = 0.500, gram percent

4. *Steenbock rachitogenic diet:* #2965

	grams
Yellow corn (ground)	76.0
Gluten	20.0
NaCl	1.0
$CaCO_3$	3.0
	100.0

Ca = 1.240, P = 0.250, gram percent

5. *Modified low-Ca, optimal P Diet* #2965—2.5 gm. percent $CaCO_3$ + 1.0 gm. Na_2HPO_4 (anhydrous)

Ca = 0.240, P = 0.468, gram percent

6. *McCollum rachitogenic diet:* #3143

Wheat (ground)	33.0
Yellow corn (ground)	33.0
Gluten	15.0
Gelatin	15.0
NaCl	1.0
$CaCO_3$	3.0
	100.0

Ca = 1.240, P = 0.280, gram percent

7. *Low P, wheat-starch diet:* #75A

Wheat (ground)	20.0
Gluten	10.0
Casein	5.0
Starch	40.0
Butter	8.0
Lard	10.0
Yeast	2.0
Salt mixture (P-free)	5.0
	100.0

Ca = 0.662, P = 0.135 gram percent

8. *Low-Ca, wheat-casein diet:* #1A

Wheat (ground)	78.5
Casein (purified)	10.0
Butter (washed)	5.0
Olive oil	4.0
Salts (Ca-free)	2.5
	100.0

Ca = 0.050, P = 0.475, gram percent

9. Minimal-Ca diet (Ca = 0.450): diet 1A + 1.0 gm. percent of $CaCO_3$

10. High-P diet (P = 1.780): diet 1A + 6.0 gm. percent of Na_2HPO_4 (anhydrous)

TABLE 2

Incidence of dental caries according to age of animals and to various diets used

(Figures in parentheses indicate number of animals in group above 100-days old and respective difference in percentage from entire group. Note prevalence of caries in groups on corn diets, and absence of such lesions in those on diets low in, or devoid of, corn.)

DIET		NUMBER OF RATS																TOTAL	PERCENT CARIES
High fat Casein*	+																		0
	−				26													26	
Sherman Corn	+					3												3	100
	−																		
Sherman Wheat	+																		0
	−					6												6	
McCollum Stock	+								8									8	13.8
	−								53									53	
McCollum #3143	+			I															12.5
	−			I		5										I		8	
Low-P Wheat	+																		0
	−			3		101	8											112	
Low-Ca Wheat	+																		0
	−		43	27	176	7	8											261	
Steenbock Low-Ca	+					4												4	50
	−					4												4	
Stock + Na_2HPO_4	+				5	8	4	5										22	48.8
	−				9	3	7	4										23	
Steenbock Vitamin D†	+		6	6		48												60 (54)	58.8 (81.8)
	−		30		8	4												42 (12)	
Steenbock†	+			3	27	4	I											35	30.1 (70)
	−		66	3	II	I												81 (15)	
Stock Vitamin D	+		I	5	6	4	II	31		6	3	19	I	10	5	2	2	108 (107)	65.4 (76)
	−	4	19	10	9	I		2		I	I	4	I	2	I	I	I	57 (34)	
Stock	+			6			12	5	22				95					141	60
	−			18	25		4	6	3				39					95	
																		1,150	
Days		0	100		200		300		400		500		600		800		1,200		

*These heads were obtained through the courtesy of Dr. W. M. Cox, Jr. A preliminary report of his investigation, "The nutritive value of pure fatty acid esters," appeared in *J. Biol. Chem.*, *97:* p. xxxvii, 1932.

† Rachitogenic.

For that purpose forty-five rats were placed on the stock diet with 1.0 gram Na_2HPO_4 (P = 0.218) percent, from weaning until they were 169 to 315 days old. But, in spite of the increased P intake, 22 of the 45 animals in this group (nearly 50 percent) had dental caries.

In the next experiment, the effects of an optimal-P, low-Ca corn diet on the development of dental caries were ascertained, since it has been alleged by some (3) that in contrast to low-P diets, rations low in Ca do not predispose to dental decay. Accordingly, the usual 3.0 grams of $CaCO_3$ in the Steenbock rachitogenic diet were reduced to 0.5 gram percent and, instead, 1.0 gram percent of Na_2HPO_4 (anhydrous) was added. The approximate Ca and P contents of this low-Ca, optimal-P corn diet were about 0.240 and 0.468 gram percent, respectively. This diet, without vitamin D, was given to four rats and the same diet with 2 percent of cod-liver oil to another group of four, from the time of weaning until the animals were 238 days of age. Two rats in each group had extensive gross dental caries while the other two showed no gross changes.

The hypothesis that the coarse corn particles, and not necessarily the paucity in Ca or in P in the diet, were responsible for the high incidence of dental caries, was further tested by making two stock diets nearly identical in their Ca and P contents (Ca, 0.500; P, 0.500) but varied in cereal grains. In one, the bulk of the diet was composed of coarsely ground yellow-corn; the other, of ground wheat (modified Sherman stock-diet). The diets were complete with respect to their mineral and vitamin contents, excepting that no vitamin D was added (*table 1*). The respective diets were given to two groups of rats of three each from weaning until 229 days old. An additional group of three rats was given the wheat diet, with 20 grams of washed beach-sand to each 100 grams of diet, with a view to abrading and injuring the enamel surface and thereby causing dental impaction and decay. This result, however, did not occur and these animals, as well as those receiving the wheat diet without sand, remained free from dental caries. In contrast, all those on the corn diet had extensive decay in their lower molars.

So far, the evidence pointed toward the idea that the high-corn content of our stock diet was a predisposing factor for molar caries, and that diets devoid of, or low in, this cereal grain were not conducive

to dental decay. Since the McCollum stock-diet contains only 20 percent of corn instead of about 60 percent in the Steenbock-Bills stock-diet, 61 animals were kept on the former diet, from weaning until they were 360 days of age. Only eight animals (13.1 percent) had caries as compared with 62.0 percent in the Steenbock-Bills stock-diet group, although both diets contained approximately the same amounts of Ca and P.

The experiment was extended to the McCollum high-Ca, low-P diet (#3143), which contains but 33 percent of ground corn as compared with 76 percent of the Steenbock diet (#2965). Of the eight animals on this diet having moderate additions of vitamin D, from weaning for 122–840 days, only one had dental caries. This result was in contrast to the high incidence in the groups on the Steenbock diet, with and without vitamin additions for similar periods of time, although the Ca and P contents of the two diets were nearly identical. A similar low incidence of dental caries in swine fed the McCollum diet (#3143) for more than a year was previously noted by Klein and associates (11).

DISCUSSION

When our data are summarized with respect to diet, and the absence and presence of caries are tabulated according to the ages of the rats (table 2), certain facts become evident: (a) Irrespective of diet, caries does not seem to be very prevalent before the animals are 100 days of age, or have been on the diets for at least 60–65 days. (b) Caries is very prevalent on diets composed chiefly of ground corn, and very infrequent on rations made of casein or of cereal grains other than yellow corn, regardless of the Ca, P, and vitamin D intakes. (c) On diets conducive to caries-like lesions, vitamin D has very little preventive influence, since caries appeared as readily and as frequently on the corn diets with vitamin D as without it. With respect to the relation of age to the incidence of dental caries in rats, it is obvious that a certain time has to elapse before pathologic changes develop. Hence, in comparing effects of other factors in the experimental production of dental caries, the time element should be considered. The data in regard to the prevalence of dental caries in the various dietary groups clearly indicate that, aside from the deficiencies

in lime salts and vitamins, the physical character of the diets determines the absence or presence of dental injury and decay.

These results are in harmony with those of Hoppert, Webber, and Caniff (10), who reported a high incidence of dental caries in rats on a stock diet containing 60 percent of coarsely ground corn, and the absence of such lesions in animals on a similar diet in which the corn was replaced by oatmeal. Attracted to the possibility that the large size of the particles of the corn in the ration might be the cause of the caries, they substituted for the coarsely ground cereal finely ground corn, capable of passing through a 60-mesh screen. On this modified ration caries did not occur. Hoppert explained the development of caries on the stock corn-diet as due to the large size of the corn particles which became lodged in the dental grooves, usually in the lower molars, and caused the development of small cavities, which encouraged more impactions of food and subsequently more extensive dental decay. Their results with the finely pulverized corn diets were recently confirmed by Klein and McCollum (12). However, the possibility that deficiencies in Ca, P, or vitamin D might be responsible for dental caries was not considered by Hoppert and his collaborators, and their studies do not apply to that phase of the problem.

The effects of deficiencies in Ca, P, and vitamin D on tooth structure were recently investigated by Agnew, Agnew, and Tisdall (3). They first placed 365 newly weaned rats on two *adequate* diets for periods varying from two to eighteen months. The first diet was composed chiefly of whole wheat, casein, milk powder and alfalfa; the second, of casein and starch. Neither diet contained corn. On these rations, not one of the 365 rats developed dental caries. In the next experiments, the investigators tried the effect of a modified Steenbock rachitogenic diet deficient in P and vitamin D, but adequate in Ca, and extremely rich in "finely-ground" whole yellow-corn (77 percent). Thirty-six rats on this diet, for periods varying from two to seven and one-half months, all developed dental caries. The addition of vitamins A, B_1, B_2, C, and E to the above diet did not protect against dental caries. In the third experiment, the above diet was fortified with 1.5 grams percent sodium pyrophosphate, and fed to eight rats for periods of from three and three-quarter to six months. None of

the eight animals developed outspoken macroscopic caries, and only two showed outspoken microscopic caries. In the fourth experiment the deficiency in vitamin D of the Steenbock, low-P diet was corrected through the addition of 2 percent of viosterol 1-D. Thirteen of the nineteen test rats on the diet for from two and one-half to five months showed "macroscopic" or "microscopic" caries. They then repeated the experiment on fifteen additional rats, keeping the animals on the diet, however, for a longer period—from six and one-half to eight months. All fifteen showed macroscopic caries. Finally, they tested a diet (#8) which did not contain ground corn, but was low in P and vitamin D. This diet was composed of cornstarch, 62 (percent); casein, 18; crisco, yeast, Osborne and Mendel salt-mixture without P, and occasional additions of fresh spinach or dried alfalfa. Ten rats on this diet, for periods of five and one-half to thirteen months, failed to exhibit macroscopic caries but showed microscopic caries in one-half the cases. When vitamin D was added to this diet, and given to nine rats for similar periods, none showed evidence of caries.

From these results, the Agnews and Tisdall concluded that, in the rat, an adequate supply of P is an important factor in the prevention of dental caries, and that an adequate supply of vitamin D prevents or delays the onset of such lesions. In this animal, they thought, the rôle of vitamin D was not of such great importance as P, although they *did* state that "it is quite possible, of course, that the consistency of the food might also be a factor in the production of caries in these animals, because the corn diets impacted much more readily than did diet #8."

To the reader the results of the experiments of the Agnews and Tisdall afford a most striking demonstration of the power of diets largely composed of ground corn to produce dental caries in the rat. Thus, of a total of 383 of their rats on *cornless* diets, "macroscopic" caries did not develop in a single instance, and "microscopic" caries occurred only in five animals (1.3 percent). On the other hand, among 121 rats on the *corn*-containing diets, counting all animals with doubtful caries as in the caries-free group and including all in which vitamin-D and P deficiencies in the diets had been corrected, 112 rats (93 percent) showed dental caries, i.e., *only 7 percent escaped*. In this

respect their results are in harmony with those of Hoppert and associates, and of our experiments. The efficacy of dietary P in preventing tooth decay cannot be evaluated from the experiments of the Agnews and Tisdall, since in their experiment, in which sodium pyrophosphate was added to an otherwise low-P ration, only eight animals were used.

The results obtained experimentally in rats also do not seem to warrant the conclusion that dental caries in this species can be prevented by an adequate intake of vitamin D. Our own data, and those of Blackberg and Berke (13), and also the results of the Agnews and Tisdall just cited, indicate little if any protective influence by vitamin D. Thus, of 50 in our series, one hundred days or older, on the Steenbock rachitogenic diet *without* vitamin D, 35 (70 percent) had dental caries; and of 66 rats of similar ages on the same diet *with* vitamin D, 54 (81.8 percent) had dental decay. On a similar diet, but lower in Ca, the Agnews observed dental caries in 28 out of 34 animals (82.8 percent)—in spite of liberal additions of vitamin D to the ration. Evidently this slight difference in incidence might have been due to variations in the size of the particles of the ground corn, rather than to the presence or absence of vitamin D, in the food. The ineffectiveness of vitamin D for the prevention of caries-like lesions in rats, as induced by *corn* diets, is also evident from the fact that dental caries was as prevalent among the rats on the corn stock-diet, with vitamin D additions as without them, as may be seen from *table 2*. That vitamin D, *per se*, does not damage dental tissue is evident from the fact that caries was absent in our rats on casein or wheat diets and receiving large doses of viosterol for relatively long periods of time.

From the facts just enumerated—the occurrence of caries-like lesions in rats on corn diets, and their absence on rations poor in this grain regardless of the Ca, P, and vitamin D intakes—it is evident that results on the production of caries-like lesions in rats cannot be transferred *in toto* to the problem of dental decay in human beings. While it is undoubtedly important that the human dietary should be "complete," not only for the preservation of the teeth, but also for the maintenance of health and the prevention of other maladies, it is equally important to abstain from basing conclusions on incomplete

experimental evidence. Such conclusions result only in therapeutic fads and detract from an understanding of the issue at hand. It is only through better controlled experimentation on animals, and observations on human beings, that the problem of the causation and prevention of dental decay in man can be clarified. Studies of the effects of vitamins and diets on dental structure and caries, in growing

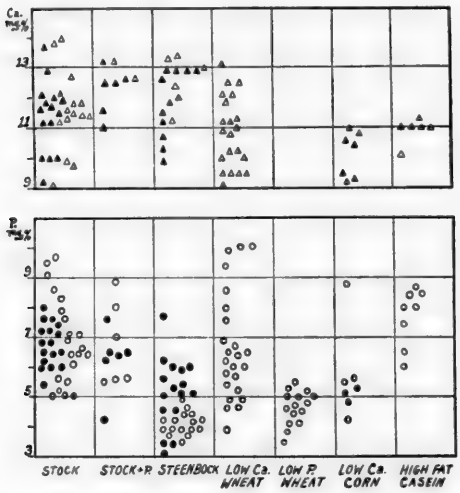

FIG. 1

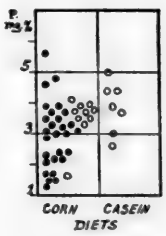

FIG. 2

FIG. 1. LACK OF RELATIONSHIP BETWEEN INCIDENCE OF DENTAL CARIES AND LEVELS OF SERUM Ca AND INORGANIC P IN RATS

Shaded triangles and circles indicate presence of caries; unshaded ones, absence of caries.

FIG. 2. LACK OF RELATIONSHIP BETWEEN LEVELS OF SERUM INORGANIC P AND INCIDENCE OF DENTAL CARIES IN RATS DESCRIBED BY AGNEW, AGNEW AND TISDALL (3)

Shaded circles indicate presence of caries; unshaded ones, absence of caries

children, are now in progress in many clinics. The systematic administration of vitamin D for the prevention of rickets, now practised in most populated communities in the United States, offers an excellent opportunity for study of effects of vitamin D on dentition. Indeed, results of studies in this direction have been recently reported by Eliot and her associates (14) in New Haven, and similar programs have been initiated by Hess, and by Schoenthal and Brodsky

in New York, and by us in Baltimore in connection with our clinic for the study of rickets.

We do not wish our results to be interpreted as minimizing the value of adequate intakes of Ca, P, and vitamin D in the prevention of tooth decay in human beings, since their place in the diet is now well established. We do, however, desire to call attention to the fact that the caries-like lesions produced in rats by *corn* diets probably have no counterpart in the genesis of dental caries in man, as already pointed out by Rosebury (15); and for this reason, the efficacy of diet and of vitamins in the prevention of caries in human beings cannot be ascertained from such experimental observations. That the Ca, P, and vitamin D intakes play little, if any, rôle in the prevention of dental caries resulting from feeding *corn* diets to rats may also be surmised, indirectly, from a study of the concentrations of the serum Ca and inorganic P in the various experimental groups. These are shown graphically in *fig. 1.* The values for serum inorganic P are especially interesting because they indicate that the "blood phosphorus critical level" of 10.5 ± 0.5 milligrams percent, as suggested by Klein and McCollum (2), is too high for apparently normal rats, whether caries happens to be present or not; and that the concentrations of the inorganic P in the serum do not seem to bear any definite relationship to the presence or absence of caries (*fig. 1*), since caries was present in animals whose concentrations of inorganic P in the serum were high and absent in others in which the concentrations were low. Also, in our experiments no definite relationship seemed to exist between the levels of serum Ca and the incidence of dental caries.

The data for inorganic P reported by Agnew, Agnew and Tisdall (3) for rats subjected to various diets, protective or conducive to tooth decay, are in accord with our own observations. Their results, arranged graphically, are shown in *fig. 2.* It is seen that the concentrations of serum inorganic P, even in the animals which were free from dental caries, are below 6.0 milligrams percent; and also that, as in our experiments, the presence or absence of caries does not bear any definite relationship to the levels of inorganic P in the serum. A similar lack of relationship between the concentrations of the serum Ca and inorganic P to the incidence of dental caries in pregnant

women was recently reported by Mull and Bill (16). It is also interesting to note that in the experiments of Hoppert, and the Agnews and Tisdall, the percentages of bone ash, which presumably indicate the degree of Ca and P assimilation and the efficacy of vitamin D, bear no relation to the presence or absence of caries.

No definite conclusion can be drawn from our data as to the relation of sex to the incidence of caries. Since the incidence of caries in rats on corn diets depends more on mechanical injury by hard particles of food than on defective calcification, which might result from reproduction and lactation, it is impossible to ascertain whether the presence or absence of caries is due to a definite sex factor or merely to chance. From our meager data, it would seem that there is very little difference in the incidence of caries in the two sexes. Thus, of 602 rats, more than one hundred days old, on diets predisposing to tooth decay, 65.4 percent of the females (212 out of 324) and 59.7 percent of the males (166 out of 278) had dental caries.

SUMMARY

An analysis of the incidence of caries-like lesions in 1150 rats, on diets of various compositions for a period of 130 to 1153 days, revealed that

(1) Caries is infrequent in rats below the age of one hundred days, irrespective of the diet.

(2) The incidence of caries is high in rats on diets containing a large percentage of coarsely ground *corn*, and absent on diets composed of casein and starch, or of grains the particles of which are finer and softer than corn.

(3) In the rat there is no correlation between the Ca and P intakes, the concentrations of Ca and inorganic P of the serum, and caries-immunity and caries-susceptibility.

(4) Since the caries-like lesions in the rat were probably due to physical injury by corn particles in the diet, the efficacy of vitamin D in preventing tooth decay cannot be evaluated from such experiments.

(5) Results on the experimental production by this means of caries-like lesions in rats are not necessarily applicable to the etiology of dental caries in human beings.

REFERENCES TO LITERATURE

(1) LENNOX, J. *Brit. Dent. J., 52:* 570, 1931.
(2) KLEIN, H., AND MCCOLLUM, E. V. *Science, 74:* 662, 1931.
(3) AGNEW, W. C., AGNEW, R. G., AND TISDALL, F. F. *J. Am. Dent. A., 20:* 193, 1933; and *J. Pediatrics, 2:* 190, 1933.
(4) SHOHL, A. T., AND BENNETT, H. B. *J. Biol. Chem., 73:* 633, 1928.
(5) SHELLING, D. H. *J. Biol. Chem., 96:* 229, 1932.
SHELLING, D. H., AND ASHER, D. E. *Bull. Johns Hopkins Hosp., 50:* 318, 1932.
(6) STEENBOCK, H., AND BLACK, A. *J. Biol. Chem., 64:* 263, 1925.
(7) KLEIN, H., AND SHELLING, D. H. *J. Dent. Research, 11:* 151, 1931.
(8) SHELLING, D. H., AND ASHER, D. E. *Bull. Johns Hopkins Hosp., 50:* 344, 1932.
(9) BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. *J. Biol. Chem., 90:* 619, 1931.
(10) HOPPERT, C. A., WEBBER, P. H., AND CANIFF, T. L. *Science, 74:* 77, 1931; also *J. Dent. Research, 12:* 161, 1932.
(11) KLEIN, H., MCCOLLUM, E. V., BUCKLEY, S., AND HOWE, P. E. *J. Am. Dent. A., 17:* 782, 1930.
(12) KLEIN, H., AND MCCOLLUM, E. V. *J. Dent. Research, 13:* 69, 1933.
(13) BLACKBERG, S. N., AND BERKE, J. D. *J. Dent. Research, 12:* 609, 1932.
(14) ELIOT, M. M., SOUTHER, S. P., ANDERSON, B. A., AND ARNIM, S. Read before the American Pediatric Society, Washington, D. C., May 9, 1933.
(15) ROSEBURY, T. *Arch. Path., 15:* 260, 1933.
(16) MULL, J. W., AND BILL, A. H. *Proc. Soc. Exper. Biol. a. Med., 30:* 854, 1933.

STUDIES, IN THE RAT, OF SUSCEPTIBILITY TO DENTAL CARIES

III. The Experimental Production of Typical Dental Fissure-caries and Other Lesions in Rats, and Preliminary Studies of Their Etiology[1]

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It has been noted elsewhere (7) that the report of Shibata (10) on experimental dental caries in rats differs in important details from those of other workers. Shibata described lesions beginning in the molar fissures of young rats. Lesions indicated by other workers have been clearly recognizable as destructive processes that occur for the most part in older animals at the molar-cusp summits (where dentin is normally exposed in the rat)[2] or they have been inadequately described. Shibata's description appears to correspond more closely to dental caries in man than cusp lesions in a region that has no true analogue in human teeth. In view of our previous unsuccessful attempts to produce dental caries in rats (8, 9), it appeared advisable to repeat Shibata's work in an effort to determine whether the rat is susceptible to a type of dental caries comparable to that of man. The successful accomplishment of this objective has opened a promising new field for investigation into the etiology of the lesion. Our preliminary findings in this field are presented herewith.

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[2] See fig. 1.

EXPERIMENTAL

Shibata states that he reared young rats on a diet consisting of "whole" rice and greens, plus 5–10 percent of various carbohydrates, of which dextrin and maltose were most effective in producing caries. The lesion, he states, developed in the molar fissures in about twenty days, and destroyed the crown in a week. Our first experiment was an attempt to repeat this finding. The animals were the Long-Evans strain of hooded rats, originally given to us in March, 1930, by Prof. P. E. Smith of the Department of Anatomy, and since bred by ourselves on a modified McCollum stock-ration (9). Full litters were distributed among the experimental groups in all cases at weaning, twenty-two days after birth. All animals were weighed every five days. Diets, other than spinach, were fed *ad lib.*

Group I. Four litters, comprising 22 animals, were placed on Diet 1: Brown rice ("Comet"), unground, 92 (percent); white-potato dextrin, 8; fresh spinach leaves, 5 grams per rat per day. In accordance with information available at this time (11), the animals were divided into two groups, one of which (Ia) received water *ad lib*; the other (Ib), no water excepting that on the spinach leaves, which were washed and drained before feeding. These animals were killed in litter-mate pairs at intervals of about a week after 20 days on the diets. Unstained ground sections were prepared from one side of the mandible of each, and twelve or more 20–30μ celloidin sections, stained with hematoxylin and eosin, from the other side. Typical dental fissure-caries and other lesions were produced on both diets. Details of this finding are given below. The second experiment, initiating a detailed etiological study, was a test of the influence of rice particle-size and consistency on the production of these lesions. The work of Hoppert, Webber, and Canniff (5) has given prominence to this matter, although at this writing the precise character of the lesions obtained by these workers has not been established.

Group II. Six litters, comprising 45 animals, were distributed among four groups on the following diets, respectively:

Diets	*2* Percent	*3* Percent	*4* Percent	*5* Percent
Brown rice	Unground, 90	10-mesh, 90	20-mesh, 90	Cooked, 90
White-potato dextrin	5	5	5	5
Maltose	5	5	5	5

Fresh spinach leaves, 3 grams per rat per day in all.

The rice was ground by hand in a "Miag" mill, the 20-mesh rice representing the finest product obtainable by this method. In each case particles that

did not pass the sieve were reground until they did, so that each product represented all of the original rice. For Diet 5 a weighed quantity of rice was added daily to five times its weight of water, and cooked in a double boiler for 1 hour. All of the water was absorbed by the rice grains, and the mixture was not thereafter washed or drained. Dextrin and maltose were added in accordance with the dry weight of the rice. In this way changes in composition of the diet other than those produced by heating were avoided. Growth on Diet 5 was slightly better than on the others, and the mortality was lower, apparently because more of Diet 5 was ingested. Examination of the feces of animals in Group II failed to reveal undigested rice particles in any case, and the iodine test for starch in the feces was negative. Defective calcification of teeth and bone, and characteristic slight pulp changes, occurred in all the animals of the group in about equal degree, the diets evidently being about equally deficient.

The results of these experiments indicated a relation between dental-caries production and the physical character of the diet. To test this finding more conclusively, one litter of nine animals was divided into two groups on the following diets:

Diets	*22* Percent	*23* Percent
Brown rice	100-mesh, 92	10-mesh, 92
White-potato dextrin	8	8

Fresh spinach leaves, 5 grams per rat per day.

The 100-mesh[3] rice was ground under our supervision through the courtesy of S. B. Penick and Co.[4] from "Comet" brown rice supplied by us. The grinding, as before, was repeated until the entire product had passed the sieve. All of the foregoing diets are markedly deficient. To evaluate the importance of the deficiency, it was necessary to study the effect of comparable adequate diets that embodied as far as possible the physical character of the caries-producing ration. This was done in the succeeding group.

Group III. Twenty-four members of six litters were used in this experiment. These animals, in two groups, were placed on the following diets, respectively:

Diets (*continued on next page*)	*6* Percent	*7* Percent
Brown rice, 10-mesh	76	68
White-potato dextrin	4	8

[3] The term "mesh" is used in this connection to refer to a product all particles of which passed through a sieve having the stated number of openings per linear inch.

[4] Factory, Weehawken, N. J.

Diets (continued from preceding page)	*6* Percent	*7* Percent
Maltose	4	8
Wheat gluten	10	10
Osborne-Mendel salt mixture: IV	4	4
Cod-liver oil	2	2
Fresh spinach leaves, 3 grams per rat per day.		

Growth on these diets was less than optimal at first, apparently because of the change from the stock ration on which the litters were bred. This was evidenced by the following additional test: Three of the lighter males were removed from each group after 36 days and given Diets 14 and 15, modified, respectively, from Diets 6 and 7, as follows:

Diets	*14 (from 6)* Percent	*15 (from 7)* Percent
Brown rice, 10-mesh	66	68
White-potato dextrin	4	8
Maltose	4	8
Wheat-gluten	10	10
Egg albumin	10	—
Osborne-Mendel salt mixture: IV	4	4
Cod-liver oil	2	2
Yeast vitamin: Harris powder	0	0.4 gram per rat per day
Fresh spinach leaves, 3 grams per rat per day in both.		

These additions improved growth slightly, but most of the animals on diets 6, 7, 14 and 15 reached an optimal growth-level within the 100-day period, as is shown by *table 1*, where the values for stock-ration animals are those obtained in a previous investigation (9). These diets contained the following percentages of Ca and P:

Diet	*Ca*	*P*	*Ratio*
6	0.59	0.55	1.1
7	0.59	0.53	1.1
14	0.59	0.52	1.1
15	0.59	0.53	1.1

The animals were normal and healthy in appearance, and their dental and osseous tissue-structure could not be distinguished from that produced on the stock ration, which we previously accepted as our normal standard.

Group IV. The data reported for this group are part of a long-time experiment still under way (February 1933), on an adequate diet, the Ca level being higher than in Group III. The experiment tested the action of

particle size. Diet 16 contains 10-mesh rice; diet 18, 100-mesh rice; their composition, otherwise identical, being (percent): Brown rice, 70; white-potato dextrin, 8; wheat-gluten, 10; egg albumin, 4; Osborne-Mendel salt mixture: IV, 4; calcium lactate, 2; cod-liver oil, 2; yeast vitamin: Harris powder, 0.4 gram per rat per day; fresh spinach leaves, 3 grams per rat per day. These diets contain 0.85 percent of Ca and 0.53 percent of P; Ca:P = 1.6.

TABLE 1

Average growth of animals on diets 6, 7, 14, and 15 and of stock ration animals

DIET	NO. OF RATS	DAYS FROM WEANING										
		0	10	20	30	40	50	60	70	80	90	100
		gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Stock ration, Males	2	29	52	92	124	144	154	185	195	212	216	235
6, Males	4	40	50	71	80	105	124	146	166	185	207	—
7, Males	4	41	51	72	83	102	118	150	177	197	222	235
14, Males	3	35	46	65	76*	99	126	157	191	207	215	240
15, Males	3	34	42	61	68*	95	118	148	180	202	216	250
Stock ration, Females	2	36	58	98	120	140	151	161	166	174	176	180
6, Females	5	33	48	70	84	105	125	140	153	160	175	183
7, Females	3	32	36	34	48	72	85	103	116	129	—	—

* Diets 14 and 15 started between 30th and 40th days.

RESULTS

Three distinct types of dental lesions have been obtained in this work (*fig. 1*). One begins in the enamel at the base of the molar fissures. A second results from fracture of the cusp, and a third from a process of dissolution resembling caries of dentin. The second and third begin in the dentin at the cusp summit. Of these lesions only the first appears comparable to human dental caries. Cusp "caries," the third type, occurred in only a few instances; was scattered so widely among the groups as to permit no correlation with experimental conditions; and accordingly no further mention of this lesion will be made.

In animals on the experimental diets for less than about 100 days, true caries was rarely recognizable macroscopically, since little or no gross cavitation had occurred up to this time, although the process might have become sufficiently deep to produce active pulpitis.

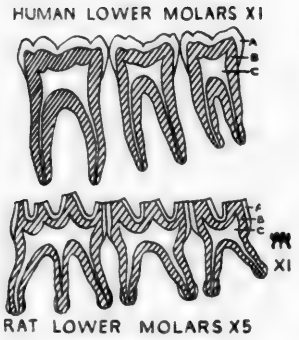

FIG. 1

FIG. 2

FIG. 1. COMPARISON OF LOWER MOLARS OF MAN AND RAT (SCHEMATIC)

A, enamel. *B*, dentin. *C*, pulp. Note general similarity of tissue relationships. Three differences are shown: Dentin is exposed at summit of rat molar cusps, whereas enamel covers entire crown of human molars. Occlusal fissures are relatively deeper and wider in rat molars (actually they are similar in size in the two species). Rat molars approximate over a surface rather than at a point, as in human molars. This last difference may account for relative infrequency of interproximal caries in rat.

FIG. 2. APPEARANCE OF EARLY FISSURE CARIES, *A*, AND CUSP FRACTURE, *B*, IN GROUND SECTION

Discoloration at *A* has been accentuated by photographing through blue filter. Note pulp abscess at *C*. Rat No. 211. Deficient diet (Ib), 40 days. Ground section, mounted in glycerin, unstained. ×35.

FIG. 3. EARLY CARIES OF MIDDLE FISSURE OF FIRST MOLAR, *A*. X-RAY INSET SHOWS RADIOLUCENCY IN CORRESPONDING REGION

Rat No. 209. Deficient diet (Ib), 54 days. Ground section, glycerin, unstained. ×200.

FIG. 4 FIG. 5

FIG. 4. CAVITY IN FIRST MOLAR, EVIDENTLY RESULTING FROM FRACTURE OF SUBDISTAL CUSP. PULP WIDELY EXPOSED AND NECROTIC. INTERPROXIMAL ENAMEL CARIES SHOWS AT *A*

Rat No. 214. Deficient diet (Ia), 44 days. Ground section, glycerin, unstained. ×35.

FIG. 5. DIFFUSE PENETRATION OF ENAMEL IN FIRST STAGE OF FISSURE CARIES. DENTIN NOT AFFECTED

Rat No. 410. Adequate diet (16), 218 days. ×320.

FIG. 6 FIG. 7

FIG. 6. PENETRATION THROUGH LAMELLAE IN FIRST STAGE OF FISSURE CARIES. SPREAD AT DENTINO-ENAMEL JUNCTION MAY BE BEGINNING AT *A*. NOTE DENTINOID AT *B*

Rat No. 208. Deficient diet (Ia), 35 days. ×350.

FIG. 7. LOCALIZED SPREAD AT DENTINO-ENAMEL JUNCTION IN SECOND STAGE OF FISSURE CARIES. NOTE BEGINNING INFILTRATION OF DENTINAL TUBULES. *A*

Rat No. 316. Adequate diet (7), 81 days. ×260.

FIG. 8

FIG. 9

FIG. 8. EXTENSIVE SPREAD AT DENTINO-ENAMEL JUNCTION IN SECOND STAGE OF FISSURE CARIES. CHANGES CHARACTERISTIC OF LOW CALCIUM DIETS SHOWN IN DENTIN AND PULP

Rat No. 213. Deficient diet (Ib), 44 days. ×65.

FIG. 9. LOCALIZED FISSURE CARIES IN THIRD STAGE, *A*. NOTE NORMAL TISSUES

Rat No. 308. Adequate diet (7), 67 days. ×50.

FIG. 10

FIG. 11

FIG. 10. SAME AS FIG. 9. ×250.

Note infiltration of dentinal tubules leading from localized blue-staining zone, *A*.

FIG. 11. LOCALIZED FISSURE CARIES IN THIRD STAGE, *A*. PULP TORN AT *B* IN SECTIONING. NOTE NORMAL TISSUES

Rat No. 284. Adequate diet (15), 95 days. ×240.

This does not agree with the statement of Shibata (10) that caries destroys the rat molar in a week. It is likely that his statement was based on the occurrence of fracture lesions (p. 396). Since it was frequently impossible to ascertain the manner of origin of gross lesions, it seemed essential, for the determination of the incidence of fissure caries, to study sections of decalcified jaws from animals killed relatively early. Ground sections provided less definitive information. This was the procedure adopted throughout this work; after the completion of the first experiment, the preparation of ground sections was largely abandoned. Ground sections provided certain data, however, not afforded by decalcified material. Animals killed after 33 days on both diets showed yellowish or brownish discoloration in the enamel at the base of some of the molar fissures (*figs. 2, 3*). Radiographs of these sections revealed at these points (*fig. 3*) loss of opacity extending into dentin in some instances, apparently indicating partial decalcification. These sections also exhibited fractures, usually of the large subdistal cusp of the first molar, exposing the pulp (*fig. 2*). In other sections large cavities occurred in the same region, evidently resulting from cusp fracture (*fig. 4*).

A description of the two chief lesions under discussion, derived from a study of the decalcified sections, follows.

Fissure caries. Four stages in the progress of fissure caries may be indicated respectively, as +, ++, +++, and ++++. 1. (+) *Penetration of enamel.* The process may penetrate diffusely from the food plug toward the dentin, producing discoloration and perhaps consolidation (2) of enamel keratin, and thus accentuating its visible normal structure (*fig. 5*); or it may penetrate narrowly through one or more enamel lamellae, reaching the dentin with little apparent effect on the surrounding enamel matrix (*fig. 6*). The latter type of penetration seems to depend only on the presence of enamel lamellae at the site of the lesion. Each type has been observed on both deficient and adequate diets. 2. (++) *Spread at the dentino-enamel junction.* The process, having reached the junction of dentin and enamel, spreads along this plane as a densely blue-staining deposit, replacing or resulting from normal dentin substance. This lateral spread may be localized (*fig. 7*) or extensive (*fig. 8*). To some extent the degree of spread seems to depend on the state of calcification of the dentin, a wider

spread occurring in general on deficient than on adequate diets (*figs. 7–13*). The nature of the deposit has not been determined. In our relatively thick sections, it appears homogeneous in structure under all magnifications up to 1500 diameters, but at its free margin the

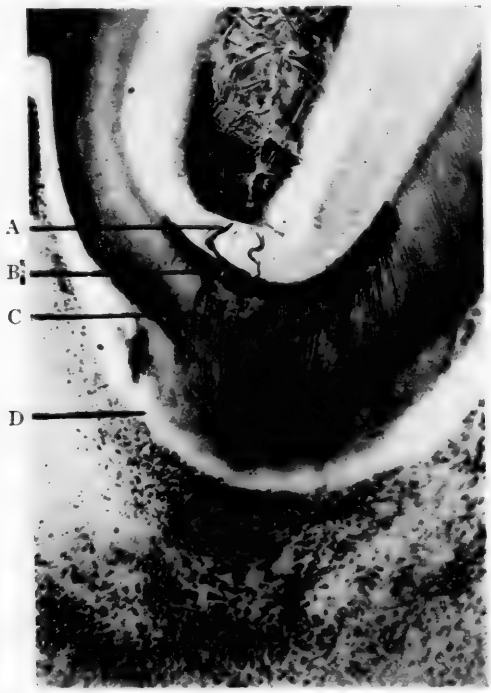

FIG. 12. FISSURE CARIES, THIRD STAGE, WITH PULPITIS. NOTE PATH OF LESION THROUGH ENAMEL LAMELLAE, *A*, EXTENSIVE BLUE-STAINING DEPOSIT AT DENTINO-ENAMEL JUNCTION, *B*, AND TUBULAR INFILTRATION IN DENTIN, *C*. PLANE OF SECTION SLIGHTLY OFF CENTER OF LESION. DENTINOID SHOWS AT *D*.

Rat No. 212. Deficient diet (Ia), 62 days. ×160.

suggestion of a fine fibrous structure may appear under oil immersion. 3. (+++) *Penetration of dentin.* The dentinal tubules leading pulpward from the blue-staining deposit become widened and accentuated by filling with what seems to be this same substance (*fig. 12*). Under higher magnification (*fig. 13*), typical "liquefaction

foci" are seen. As this tubular infiltration approaches the pulp, pulpitis appears in characteristic fashion (*fig. 12*). 4. (++++) *Loss of surface continuity and formation of a cavity.* This final stage in the process, definitely identifiable as such, has been seen relatively

FIG. 13. SAME AS FIGURE 12. ×430.

seldom in these preparations (*figs. 14, 15*). When it occurs grossly, secondary fracture and loss of undermined cusps probably occur, and it is accordingly difficult to decide whether lesions such as that shown in *fig. 16* resulted from fissure caries or from cusp fracture. The lesion in question is classed in the record as cusp fracture.

FIG. 14

FIG. 15

FIG. 14. FISSURE CARIES, SHOWING BEGINNING OF GROSS CAVITATION. PULP EXPOSURE HAS NOT OCCURRED

Rat No. 234. Deficient diet (3), 84 days. ×150.

FIG. 15. FISSURE CARIES, SHOWING BEGINNING OF GROSS CAVITATION. DENTAL TISSUES NORMAL

Rat No. 389. Adequate diet (16), 181 days. ×120.

FIG. 16

FIG. 17

FIG. 16. GROSS LESION OF SECOND MOLAR, ILLUSTRATING DIFFICULTY OF DETERMINING MANNER OF ORIGIN OF LARGE CAVITIES

Rat No. 217. Deficient diet (Ia), 122 days. ×18.

FIG. 17. NON-CARIOUS SECOND MOLAR FISSURE, SHOWING DEFECTIVE CALCIFICATION OF DENTIN. NOTE IMPACTED FOOD PLUG, *A*, AND FRAGMENT OF ENAMEL KERATIN, *B*, REMAINING AFTER DECALCIFICATION

Rat No. 249. Deficient diet (5, cooked rice), 102 days. ×35.

FIG. 18

FIG. 19

FIG. 18. Early cusp fracture lesion with pulp horn abscess. Secondary carious changes not yet apparent. Rat No. 302. Deficient diet (9), 52 days. ×75.

FIG. 19. Cavity in first molar, evidently originating by cusp fracture, with secondary caries of dentin, *A*, necrotic pulp, *B*, and periapical abscess, *C*. Rat No. 255. Deficient diet (3), 62 days. ×30.

FIG. 20

FIG. 21

FIG. 20. Gross cavity in first molar, evidently originating by cusp fracture. Gingival caries on distal wall, *A*, probably developed by extension from larger lesion. Note disintegrated dentin at *B*. Rat No. 217. Deficient diet (Ia), 122 days. ×30.

FIG. 21. Reproduction of photomicrograph from Barker (1) of caries of human tooth. Compare with figs. 12 and 13. ×125 as reproduced.

The occurrence of fissure caries seems to be independent of the calcified state of the tooth. On deficient diets the teeth having caries also show the characteristic evidences of defective calcification (*figs. 6, 8, 12, 13, 14*). Conversely, it may hardly be necessary to point out that teeth may be calcified equally poorly without showing caries

TABLE 2

Incidence of fissure caries and fracture lesions on deficient diets

Including only rats on diets 35 days and more

GROUP	NO.	DIET	NO. ANIMALS	FIRST FISSURE CARIES	EXPERIMENTAL PERIOD	FISSURE CARIES							FRACTURE LESIONS	
						Animals		Lesions in 3 teeth per animal						
						No.	Percent	No.	Percent	+	++	+++	++++	No.	Percent
				days	*days*										
I		Unground rice, 8 percent dextrin:													
	1a	$+H_2O$	8	27	122	8	100	17	71	3	10	2	2	5	21
	1b	$-H_2O$	7	27	63	4	57	10	48	0	6	3	1	4	19
II		Rice, 5 percent dextrin, 5 percent maltose:													
	2	Unground	8	24	102	8	100	16	67	2	7	5	2	2	8
	3	10 mesh	9	41	102	9	100	19	70	1	7	6	5	9	33
	4	20 mesh	9	81	102	3	33	7	26	1	3	3	0	1	4
	5	Cooked	11	—	102	0	0	—	—	—	—	—	—	0	0
IIa		Rice, 8 percent dextrin:													
	22	100 mesh	5	—	150	0	0	—	—	—	—	—	—	0	0
	23	10 mesh	4	47	150	4	100	7	58	0	3	2	2	3	25
Total			61			36		76		7	36	21	12	24	

(*fig. 17*). On adequate diets, on the other hand, the teeth appear structurally normal in all respects, as would be expected from the character of the diets (*figs. 9, 10, 11, 15*).

Fracture lesions. The primary change in this case is fracture through the dentin, sometimes also through the enamel, of the cusp,

generally producing exposure and consequent infection of the pulp before other changes begin in the dentin (*fig. 18*). Subsequently the dentin undergoes carious changes (*fig. 19*), further destruction of the tooth ensues, and a large cavity forms rapidly (*fig. 20*). The most

TABLE 3

Incidence of fissure caries and fracture lesions on adequate diets

Including only rats on diets 35 days and more

GROUP	NO.	DIET	NO. ANIMALS	FIRST FISSURE CARIES	EXPERIMENTAL PERIOD	FISSURE CARIES								FRACTURE LESIONS	
						Animals		Lesions in 3 teeth per animal							
						No.	Percent	No.	Percent	+	++	+++	++++	No.	Percent
				days	*days*										
III	6	10 mesh rice, 4 percent dextrin, 4 percent maltose	8	93	103	1	12	1	4	0	1	0	0	0	0
	14	10 percent extra protein	3	95	105	2	67	5	56	3	2	0	0	0	0
	7	10 mesh rice, 8 percent dextrin, 8 percent maltose	7	53	100	5	71	9	43	5	2	2	0	0	0
	15	+ yeast	3	95	105	2	67	3	33	1	1	1	0	0	0
IV		Rice, 8 per cent dextrin, optimal:													
	16	10-mesh	7	100	218	6	86	13	62	6	3	2	2	0	0
	18	100-mesh	7	218	218	1	14	1	5	1	0	0	0	0	0
Total			36			17		32		16	9	5	2	0	

common site of these lesions, in the mandible, was the large subdistal cusp of the first molar, which seems architecturally to bear the brunt of masticating stress. Such cavities, when fully developed, may be seen to occupy a position directly under what had been the center

of the subdistal cusp; otherwise, as we have noted, they cannot be distinguished from cavities resulting from fissure caries. All such gross lesions in this work have been classed as fracture cavities. Obviously, although the progress of this lesion simulates true caries in its secondary stage, its manner of inception differs greatly, both from fissure caries in rats, and from dental caries in man. It also differs in etiology from fissure caries, as will be noted below.

Incidence of the lesions. The incidence of fissure caries and fracture lesions on the deficient diets is given in *table 2;* on the adequate diets, in *table 3.* Except for the entries under "first fissure-caries" for the first three diets in *table 2*, these tables are based on a minimum experimental period of 35 days—the approximate average time for the first appearance of fissure caries on the deficient diets. The values for fissure caries and fracture lesions are the numbers in a study of three molars per animal; the percentages are given on the same basis. It will be observed that all animals on Diets 1a, 2, 3, and 23 (whole or 10-mesh rice, with water), for 35 days and more, had fissure caries in one or more teeth, 58 to 71 percent of the teeth examined being affected. The incidence of fracture lesions among these animals was also relatively high. The incidence of fissure caries on Diet 1b (whole rice, water restricted) was somewhat reduced. These animals did not survive beyond 63 days on the diet; two of the last survivors, evidently suffering from dehydration and inanition, did not show caries. On Diet 4 (20-mesh rice) caries did not appear until 81 days; the three animals on the diet from this point on showed caries. On Diets 5 (cooked rice) and 22 (100-mesh rice), however, neither caries nor fracture lesions occurred—in the second instance during an experimental period of 150 days. In view of the result obtained on Diet 4, we may suspect that the absence of caries on the cooked and finely ground rice-diets may represent a retardation rather than a prevention of the lesion. It appears clear, nevertheless, that the physical character of the rice in these diets has determined the incidence of both fissure caries and fracture lesions.

On the adequate diets (*table 3*) the incidence values are in general lower, and + and ++ lesions predominated; whereas on the deficient diets, the lesions were generally more advanced. This again, however, may be an effect of retardation rather than of prevention.

Although on Diet 6 only one animal developed caries, on Diet 7 all but one animal showed the lesion after 53 days, when it first appeared; and on Diets 14 and 15 caries was absent from animals killed at 85 days, but occurred in each case in the older animals. All animals on Diet 16, after 100 days, had caries in one or more teeth, and several well-advanced lesions were observed (*fig. 14*). Diet 18 again shows the effect of 100-mesh rice. The one small lesion in the latest of these animals, killed at 218 days, may be without significance; or may indicate that caries occurs on this diet, if the experimental period is sufficiently prolonged. Fracture lesions, as the table indicates, were not observed on the adequate diets; therefore they may be distinguished sharply from fissure caries on this basis.

DISCUSSION

The type of fissure caries described above seems to be identical with fissure caries of human teeth. Each begins in the same tissue—enamel—in analogous locations, and presents the same lateral spread at the junction of dentin and enamel, and the same picture of dentinal invasion and disintegration. It is interesting to compare the rat lesions directly with a photomicrograph of caries in a human tooth, reproduced herewith (*fig. 21*)[5]. A comparison of this picture with *figs. 12* and *13*, particularly, shows the striking similarity of the two lesions.

The results of the experiments involving the use of rice of various particle-sizes and consistencies confirm the findings of Hoppert, Webber, and Canniff (5), and indicate that the physical character of the diet is important in the production in rats of both fissure caries and fracture lesions. The absence of fracture lesions on the adequate diets, as well as on the diets containing 100-mesh rice and cooked rice, suggests that these lesions result primarily from the impact of dense food particles on teeth weakened by rachitic deficiency—a mechanism clearly not comparable to dental caries in man. In rats the action of large particles in producing fissure caries may be explained—if we assume that dental caries arises by bacterial action on food particles retained at sites about the teeth—by postulating a process of forcible impaction—wedging—of such food particles into favorable regions.

[5] We thank Dr. R. Kronfeld for permission to use this picture.

Such forcible impaction may act (a) by perforating the enamel cuticle and bringing the food mass into intimate contact with enamel, and thereafter (b) by resisting the normal mechanisms that remove or replace food masses less effectively impacted. This, if true, is a new principle; and while its application to human caries is of course uncertain, it may well be deserving of consideration in this regard.

While this work was in progress we received from Prof. Shibata decalcified sections of lesions produced in his rats, a sample of the rice he used, and other information. The sections show both fissure caries and extensive lesions of the cusp-fracture type. Only slight indications of pathological calcification occur in the dentin, as compared with the more marked changes present in our animals on the deficient diets. Comparative analyses of the rice used by Shibata—a crude product, containing much extraneous material, and ground to about 10 to 15-mesh—and that by us, offer an explanation for this difference. Shibata's rice contained 380 mg. of Ca and 595 mg. of P per 100 grams. The rice we used contains 13 mg. of Ca and 250 mg. of P per 100 grams.

Our Diets 1 to 5 are markedly deficient in mineral salts, protein, and vitamin D. These deficiencies were reflected in poor growth and defective tissue-structure. But fissure caries also occurred in animals on the adequate diets in which these deficiencies were fully corrected, and which supported normal growth and tissue structure. Accordingly the conclusion seems warranted that dietary deficiency does not play a determining part in the etiology of fissure caries in rats, and that the occurrence of such lesions is not incompatible with normal health and the absence of appreciable structural defect.

The reduced or delayed incidence of caries on adequate diets, as compared with deficient diets, on the other hand, suggests that dietary deficiency, although not a determining factor in etiology, may increase the rate of the caries process, perhaps by increasing the permeability of the defectively calcified teeth. Changing the deficient diet to make it adequate, however, involved alteration in physical character as well, if only by "dilution" of the rice particles, presumably the caries-producing ingredient. This might have been a factor in the retardation of caries on adequate diets. Other coincident agencies, such as the presence, in the adequate diets, of effective protein-buffering in the

impacted food mass, may be involved. Experiments are now in course to test these possibilities.

A more complete discussion of the relation of these findings to the work of other investigators in experimental dental caries, and to dental caries in man, is deferred to a later publication. It may suffice here to point out, first, that the clear distinction drawn for the first time by this work between fissure caries and fracture lesions may help to dispel the confusion obtaining in the field of experimental caries. Secondly, these findings do not seem to conflict with the established facts regarding dental caries in man. The findings relating to the importance of the physical character of the diet agree with the observations of Wallace (12) in principle, if not in detail. The finding of retardation of caries on adequate diets may correspond with the results of Boyd and Drain (3), Bunting (4), McBeath (6), and others in child feeding experiments. Finally, the similarity between human dental caries and the rat lesions described clearly warrants a working assumption of their interrelationship.

SUMMARY

Typical dental caries of molar fissures, histologically similar to fissure caries in man, has been produced in rats, establishing the susceptibility of these animals to dental caries. Four stages in the progress of these lesions have been described: (1) penetration of enamel, through enamel lamellae or diffusely in the absence of lamellae; (2) spread at the dentino-enamel junction; (3) penetration of dentin by infiltration of the tubules and disintegration of the dentin matrix; and (4) loss of surface continuity and formation of a cavity. These lesions have been distinguished from other destructive processes in rat teeth, one of which seems to result from the impact of dense food particles on teeth weakened by rachitic deficiency, producing fracture through the cuspal dentin primarily, followed by secondary caries-like changes. The latter is not comparable to dental caries in man.

Fissure caries has been produced on diets containing uncooked-rice particles of relatively large size, and prevented on comparable diets in which the rice was cooked or finely ground. The suggestion is offered that fissure caries may result from the forcible impaction of these large particles into the molar fissures.

Fissure caries has been produced on diets deficient in protein, minerals, and vitamin D, and also on diets in which these deficiencies were fully corrected, indicating that dietary deficiency is not a determining factor in the etiology of fissure caries in rats. Caries occurred on the adequate diets in animals that were healthy and well-grown, and in teeth without appreciable structural defect.

The incidence of fissure caries was delayed on adequate diets, however, as compared with deficient diets. This may indicate that dietary deficiency plays a rôle in the acceleration of dental caries, or it may be otherwise explainable. Further experiments are in progress.

REFERENCES TO LITERATURE

(1) BARKER, F. J. 1931 *Journal of the American Dental Association, 18*, p. 17.
(2) BIBBY, B. G. 1932 *Journal of Dental Research, 12*, p. 99.
(3) BOYD, J. D., AND DRAIN, C. L. 1928 *Journal of the American Medical Association, 90*, p. 1867.
(4) BUNTING, R. W. 1929 *Journal of Dental Research, 9*, p. 291.
(5) HOPPERT, C. A., WEBBER, P. A., AND CANNIFF, T. L. 1931 *Science, 74*, p. 77.
———— 1932 *Journal of Dental Research, 12*, p. 161.
(6) MCBEATH, E. C. 1932 *Ibid., 12*, p. 723.
(7) ROSEBURY, T. 1932 *Ibid., 12*, p. 255.
(8) ROSEBURY, T., AND KARSHAN, M. 1931 *Ibid., 11*, p. 121.
(9) ROSEBURY, T., AND FOLEY, G. 1933 *Ibid.* (in press).
(10) SHIBATA, M. 1929 *Japanese Journal of Experimental Medicine, 7*, p. 247.
(11) SHIBATA, M. 1930 Personal communication to Dr. E. C. McBeath, Columbia University.
(12) WALLACE, J. S. 1927 *Lancet*, 2, 218.

INFLAMMATORY ZONES IN THE DENTINE[1]

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Preamble

A study of reactions that occur within an irritated tissue should (a) contribute to our knowledge of the agents inducing the reaction, and also (b) indicate Nature's methods of defense. Early workers in caries research acted on this premise, and certain well-known theories were formed from examinations of diseased and modified dentine. For example, the zone of dissolution lent support to the view that the agent might be an acid; while the surrounding zone of translucent tissue was early pronounced a protective zone. This translucent zone often exhibited opacity between its confines and the pulp. The nature of these two zones was given various interpretations. These differences in opinion induced the writer to develop more exact procedures for the study of these problems. Instead of relying on the limited facilities of unstained or superficially stained ground-sections, methods of intensive staining were applied. These were based on the fundamentals in the experiences of Caush (1904) and in the experiments in staining from the pulpal ends of the tubules, i.e., the pulp cavity, as developed by the writer in 1912 (1). The results were presented in a number of publications, those relating directly to caries beginning with my Denver presentation in July, 1930 (5). The described methods permitted the presentation of definite views that were essential to an understanding of the subject. For example, the experiments showed conclusively that the plug of modified dentine often called pigmented (*figs. 1* and *2*, letter *C*)—interposed between a decayed area and the pulp—was not, as contended by Fish and by Bödecker, a

[1] Work supported in part by a grant from the Research Commission of the American Dental Association. Proceedings of the Eleventh General Meeting of the International Association for Dental Research, Chicago, Ill., March 18, 1933; *Journal of Dental Research*, 1933, xiii, p. 186.

dead area containing necrosed or degenerated tubules that were segregated from the circulation solely by the secondary deposits laid down within the pulp cavity. The experiments showed, instead, that "the plug" was characteristically modified chromophobic dentine, within which hypercalcification of ground substance, attended by sclerosis of a vast majority of its fibrils, had occurred (study legends, *figs. 1* and *2*).

In the above-mentioned paper, and in several succeeding it (6, 7, 8, 9), I also submitted proof to combat conceptions of Fish, who objects to the view that a translucent zone under caries indicates that the dentine under the floor of the cavity is alive and reacting (3, p. 992), but who believes that tubules once affected at their peripheral ends "die right down to the pulp" and also, as he states, " . . . . whether the lesion be attrition, exposed dentine . . . . or early caries, in every case we see an infarct, a dead area of dentine right down to the pulp" (2, p. 600). My experiments proved that reactions to an irritant occur primarily and uniformly within the tubule. Fish continued to affirm, however, in a presentation before the Royal Society (4), that barrier formation is confined strictly to the pulp cavity by secondary dentine. I am happy to see that Fish has recently repudiated (11) his former statements that dentinal fibrils affected at their periphery become necrosed clear down to the pulp, and now agrees with my published accounts of the occurrence of protective reaction within the tubule. Although Fish is still mistaken in another important particular, which will be considered below, it now appears that disagreement concerning the occurrence of vital changes within the tubule has been reduced to narrow limits, for Bödecker, who opposed my views and upheld those of Fish, has lately convinced himself independently that Fish was in error in assuming that formed dentine was devoid of reactive powers, and that calcific barriers occurred only within the pulp cavity (12).

Inflammatory zones

The foregoing remarks were introduced to draw attention to current views on the occurrence of vital changes within the tubules, as my assumption of the presence of inflammatory zones within dentine necessarily ascribes to it powers of potential reactivity. For our purpose I accept that part of the conception of "inflammatory" which

presumes a condition of active defense by the tissue as a reaction to irritation. The different zones already mentioned may collectively be considered under response to irritation; but I conceive, under "inflammation," a more active state than the relatively inert, chromophobic, sclerosed areas described by me in recent papers. These remarks have served as an approach to the discussion of a reaction graphically described in an exhibit among the American Dental Association's Scientific Exhibits, and presented as an essay before the Research Section of the American Dental Association, at Buffalo in September, 1932 (13; see also 10; laboratory report). There I discussed the hyperchromophilic areas surrounding the translucent zones in advanced caries. The location of these zones may be learned by referring to *figs. 1* and *2*. The lettering is adapted from my Buffalo presentation. The zones revealed by staining are lateral transparent zone, *LT;* lateral opaque, *LO; D*, zone of dissolution; and *C*, zone representing the tissue between decay and pulp.

The *lateral opaque zone* differs from the opaque sclerosed dentine commonly seen in mature teeth (7) in that its tubules and ground substance do not possess the chromophobic attributes exhibited by the latter, but are even more chromophilic than the surrounding unaffected dentine. This peculiarity signifies that its tubules are open to diffusion of plasma from the pulpal circulation. In my earlier paper I assumed that the *LO* zone is in a state of partial flux, and that it marks, as the cavity enlarges, the area of metamorphosis of the normal dentine into the sclerosed forms already described. I have accordingly called it the "inflammatory zone." The tubules of the inflammatory zone lie in sound dentine throughout their course, as I also showed in the earlier paper, and which is evident from the photographs (*fig. 1*). According to my observation, the inflammatory zone is always evident where caries attacks normal dentine. When caries advances in dentine that is already sclerosed, little or no reaction is present.

At Buffalo I had little time to discuss the *C* zone, which is dentine interposed between decay and pulp. Fish and Bödecker, who now agree that calcification occurs within the tubules, are apparently unwilling to relinquish their opinion that formation of a pulpal layer of secondary dentine covering necrosed fibrils is the mode of defense,

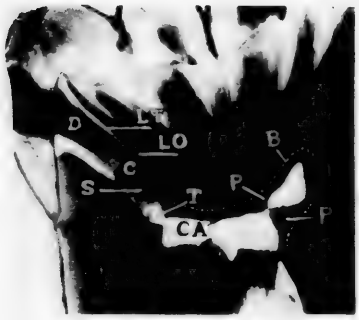

FIG. 1 FIG. 2

FIG. 1. TRANSMITTED-LIGHT PHOTOGRAPH, SHOWING PROTECTIVE PHENOMENA WITHIN DENTINE AND PULP, AS SEEN UNDER CARIES

D, zone of lysis. *C*, central zone lying between decay and pulp. *LT*, lateral transparent zone. *LO*, lateral opaque zone. *S*, opaque sclerosis within *C* zone. *CA*, pulp calcification. *B*, boundary of pulp cavity. *P*, pulp (details obliterated by stain).

FIG. 2. REFLECTED-LIGHT PHOTOGRAPH OF SPECIMEN SHOWN IN *fig. 1* AS SEEN OVER DARK BACKGROUND

Coloring of areas marked *C*, *T*, and *CA* in fig. 1 is here reversed. (For interpretation see appended "References to literature," 6, 7.)

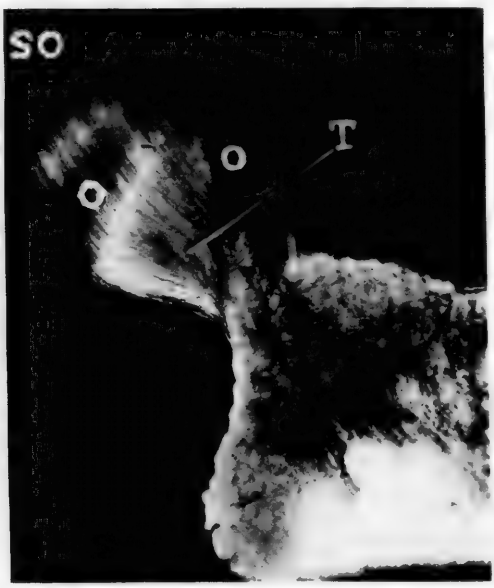

FIG. 3 FIG. 4

FIG. 3. TRANSMITTED-LIGHT PHOTOGRAPH OF AREA MARKED *T*, AND PART OF PULP CALCIFICATION SEEN IN *figs. 1* AND *2* MARKED *CA*

O, *O*, show where sclerosed, opaque dentine is intermingled with transparent dentine. Isolated tubules are stain bearing, showing that communication exists between tissue and pulp. Pulp calcification is continuous with metamorphosed dentine under decay.

FIG. 4. TRANSMITTED-LIGHT PHOTOGRAPH

M, secondarily modified primary dentine under floor of cavity containing tubules in communication with those affected by caries. *N*, normal open tubules (details obliterated by stain).

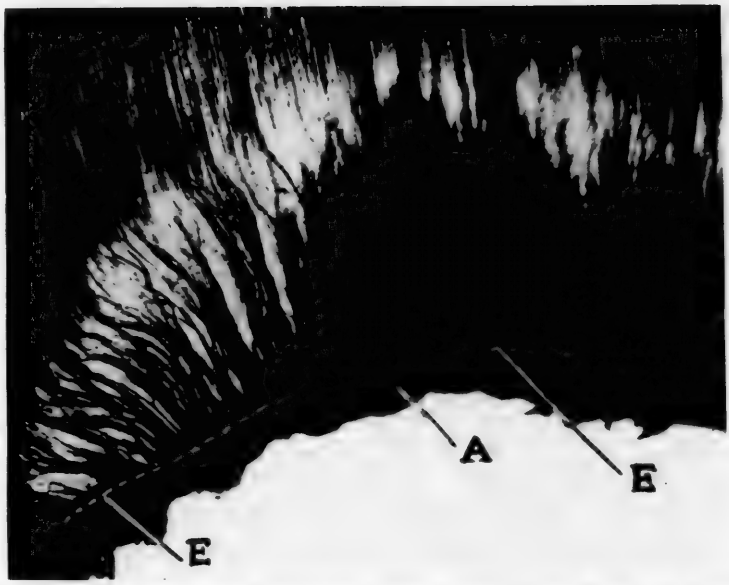

FIG. 5. TRANSMITTED-LIGHT PHOTOGRAPH: AREA SHOWN OVER *M*, *fig. 4*, HIGHLY MAGNIFIED

E, edge of dentine. *A*, pulpal surface of section with adherent stain and débris.

FIG. 6

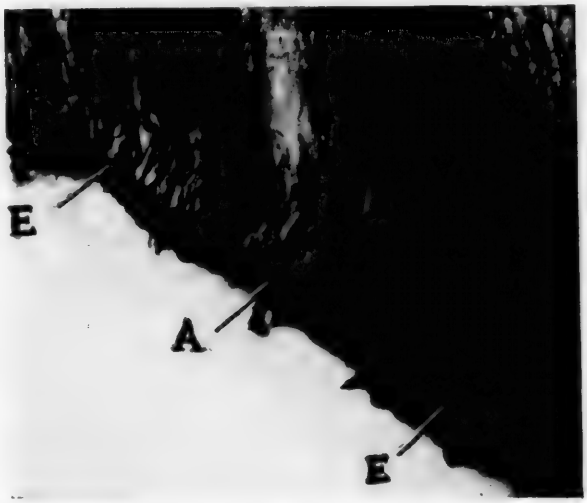

FIG. 7

FIG. 6. TRANSMITTED-LIGHT PHOTOGRAPH OF ADVANCING CARIES: ONLY SLIGHT RESISTANCE PHENOMENA IN EVIDENCE

D, zone of lysis. *C*, central zone, exhibiting open tubules in communication with pulp.

FIG. 7. TRANSMITTED-LIGHT PHOTOGRAPH: DENTINE OF *C*-ZONE SHOWN IN *fig. 6*

E, edge of dentine. *A*, pulpal surface of section, with adherent stain and débris.

which until recently constituted Fish's main argument. I have no hesitancy in pronouncing as a myth this variety of barrier-formation conceived by Fish. Before my Denver publication (5), Fish disputed the common occurrence of the translucent zone. He now contends (11, p. 356) that in 26 cases of occlusal caries in molars 23 exhibited translucent zones, and then illogically assumes that caries in all teeth anterior to the molars exhibits dead tracts, isolated from the circulation by secondary dentine. To hold that protective changes occur within the dentine in molars, but that decay in teeth anterior to them is attended by necrosed fibrils, would be to entertain a biologic incongruity. Fish still labors under the delusion that prompted his original statements. He has failed to undertake tests, suggested in my papers, convincingly proving that the so-called "dead tract" is a chromophobic sclerosed tract of opaque dentine, in all respects comparable, as an effective barrier, to the translucent zone (*figs. 3–9*). Indeed, opaque dentine is almost invariably so intimately intermingled with the translucent variety that the one commonly merges into the other by imperceptible degrees. A covering of tubule-ends, by a layer of secondary dentine, does not occur until after the fibrils so covered have undergone sclerosis. This occurred in *fig. 1*, the layer first formed having been built upon by the pulp. Secondary dentine, therefore, should not be regarded as a primary barrier, as has been done by Fish, for it is a secondary-barrier formation.

Fig. 15 in my Denver paper (5, p. 1067) showed that the pulpal ends of the tubules under caries may be open and in communication with the pulp, a condition that Fish contended was inconceivable. This view he has since corrected (11), an admission that removes the last support from his ideas of circulation within dentine. *Figs. 3*, *4*, *5*, and *6* show specimens with open tubules in the *C* zone. These are demonstrated here so that I will be understood when I state that a deposit of secondary dentine, on the pulpal walls under cavities of a size here pictured, occurs only after the dentinal tubules under the irritation have become sclerosed as indicated above. What I have contended elsewhere about the presence of isolated open-tubules in otherwise sclerosed tissue (6, 7) applies also to the zone under caries (*fig. 7*). Since some open tubules are probably always present, so long as pulp remnants occupy the crown, it is inferentially through

these that the pulp is stimulated to its protective activity in the inflammatory zone as caries advances. This view is supported by my belief that it is only in rapid decay, and where resistance is low, that the lateral transparent zone becomes perforated.

REFERENCES TO LITERATURE

(1) Beust, T. B. 1912 *Dental Cosmos*, liv, p. 659.
(2) Fish, E. W. 1928 *British Dental Journal*, xlix, p. 593.
(3) ——— 1930 *Journal of the American Dental Association*, xvii, p. 992.
(4) ——— 1931 *Proceedings of the Royal Society*, cviii, p. 196.
(5) Beust, T. B. 1931 *Journal of the American Dental Association*, xviii, p. 1060.
(6) ——— 1931 *Journal of Dental Research*, xi, p. 267.
(7) ——— 1931 *Journal of the American Dental Association*, xviii, p. 2186.
(8) ——— 1931 *Journal of Dental Research*, xi, p. 619.
(9) ——— 1932 *Dental Cosmos*, lxxiv, p. 541.
(10) ——— 1932 *Kentucky State Dental Association Bulletin*, ii, p. 17.
(11) Fish, E. W. 1932 *British Dental Journal*, liii, p. 351.
(12) Bödecker, C. F. 1933 *Dental Cosmos*, lxxv, p. 21.
(13) Beust, T. B. 1933 *Journal of the American Dental Association*, xx, p. 631.

SOME CAUSES OF VARIATION IN HARDNESS OF GOLD-COPPER CASTINGS[1]

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Study of transformations in the gold and copper alloys reveals some interesting causes of variation in the hardness of these alloys in the cast state. In the present paper we discuss variations in hardness due (a) to cavity formation, such as blowholes and shrinkage pipes; (b) to differences in composition of these gold-copper solid solutions, such as coring and segregation; and (c) to precipitation of definite compounds of gold and copper. These studies have been carried out on castings made according to specifications of the Bureau of Standards. These castings, excepting a commercial alloy that is used extensively in our Dental Infirmary but regarding the exact composition and manufacture of which little is known, were of gold and copper, alloyed in a special furnace under a hydrogen atmosphere. The castings, and all treatments of them, were conducted uniformly to reduce all variables to a minimum.

In all the castings, a definite zone of softness was located in the region of the sprue. All tests of the castings, made with the Monotron Hardness Tester, revealed a decided drop in hardness regardless of the sprueing technique. Microscopic examination of these regions showed that they contained cavities of various sizes. *Fig. 1* shows hardness curves of the commercial 22-K alloy where the sprues were placed at the ends of the castings. The larger part of each curve is more or less a straight path until it reaches the vicinity of the sprue, and then drops decidedly. *Fig. 2* shows the macrostructure of a longitudinal section of this alloy, and illustrates the porosity around the sprue. *Fig. 3* shows a hardness curve of a 40-percent atomic gold-

[1] Presented at the eleventh general meeting of the International Association for Dental Research, Chicago, Ill., March 18–19, 1933: *Journal of Dental Research*, 1933, xiii, p. 200.

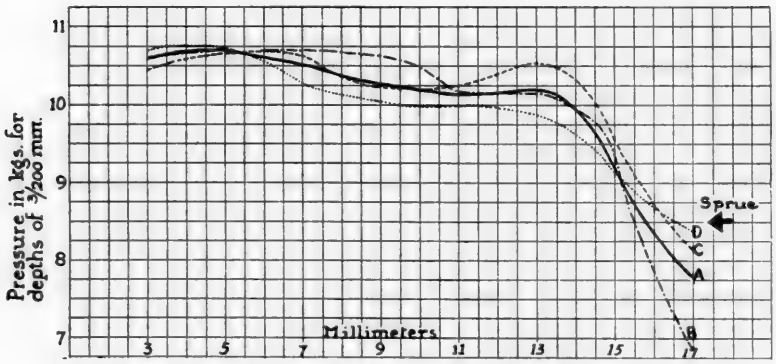

FIG. 1. GRAPH SHOWING HARDNESS CURVES OF COMMERCIAL 22-K ALLOY

A, average of all curves. *B*, hardness tests of castings in cast state. *C*, same as *B*, in annealed condition; castings heated at 600°C. for four hours and quenched. *D*, same as *C*, but slowly cooled at rate of approximately 1°C. per minute.

FIG. 2. MACROSTRUCTURE OF LONGITUDINAL SECTION OF COMMERCIAL 22-K ALLOY. ×8

Shows porosity around sprue.

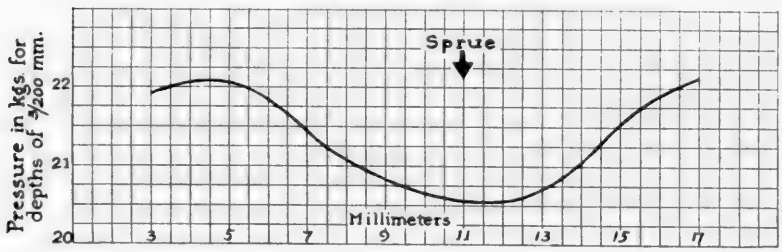

FIG. 3. GRAPH SHOWING HARDNESS CURVE OF 40-PERCENT ATOMIC GOLD-COPPER ALLOY; 67.41 PERCENT AU, 32.59 PERCENT CU, BY WEIGHT

Sprue placed toward center of body of casting.

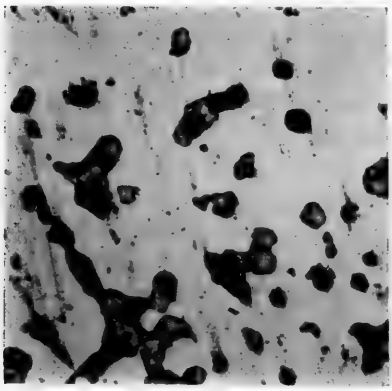

FIG. 4

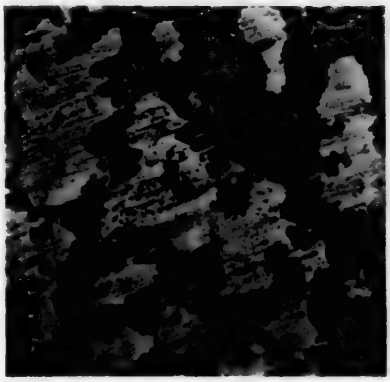

FIG. 5

FIG. 4. GOLD-COPPER ALLOY (15 PERCENT OF ATOMIC GOLD) SHOWING NON-UNIFORM DISTRIBUTION OF CRYSTALLITES; 35.58 PERCENT Au, 64.62 PERCENT Cu, BY WEIGHT. ETCHED WITH AQUA REGIA. ×150

From region at end of casting. Large white areas are gold-rich solid solutions; dark areas are copper-rich solid solutions; black spots are oxides due to high-copper content.

FIG. 5. SAME CASTING AS THAT IN FIG. 4. ×150

From region at center, showing equal amounts of gold-rich and copper-rich solid solutions.

FIG. 6

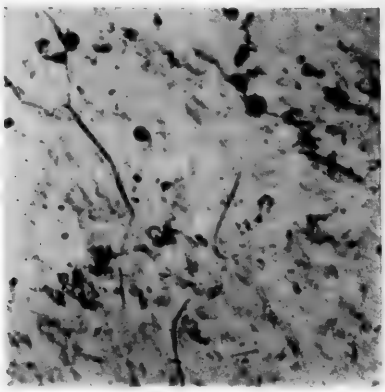

FIG. 7

FIG. 6. SAME CASTING AS THAT IN FIG. 4. ×150

From region near sprue; showing predominance of copper-rich solid solution.

FIG. 7. SAME ALLOY AS THAT IN FIG. 4; ANNEALED OR HOMOGENIZED AT 875°C. ×150

Shows complete diffusion of gold-rich and copper-rich solid solutions.

copper alloy (67.41 percent Au, 32.59 percent Cu, by weight) where the sprue was placed toward the center of the casting, the area of decreased hardness again sloping toward the point of sprueing. When the sprue was placed in the center instead of the end, the porosity was more diffused and involved more surface area. This was further substantiated by hardness tests, this area having been more extensive (*fig. 3*), a larger amount of the curve being involved in descent. In spite of all technical precautions, which however have aided considerably in reducing this condition, castings always have this area of porosity. The causes of porosity are twofold; namely, liberation of gases as the molten metal cools, and shrinkage or contraction of the metal on solidification.

The solubility of gases in liquid metals increases with rise in temperature in the melting operation. Dissolved gases are liberated as the metal cools in the mould, some escaping from the mould and some, entrapped in the casting, causing blowholes. Crystallization begins at the end of the casting and at the sidewalls. The liquid metal is pressed by centrifugal or hydrostatic forces, or by gravity, between the forming crystallites, or dendrites, until the sprue crystallizes and supply of molten metal is cut off. Then, as crystallization proceeds, there is no more free molten metal to pass between the crystallites, and the familiar shrinkage-cavities, or piping adjacent to the sprue, results. Considerable escaping gas is entrapped at this region. Blowholes such as these, when located in the interior of a casting, are less harmful than those near the periphery. The forces exerted upon a restoration during mastication at the point of contact with adjacent teeth, and upon occluding surfaces, break the surface blowholes, thus pitting the surface and subjecting the latter to further roughening by oxidation processes. Food débris clings to this area, forming a nidus for bacterial growth that may subject the same tooth or adjacent teeth to carious processes—or may harbor the tenacious bacteria of Vincent infection, thus making the eradication of the disease more difficult. This evidence shows that the point of sprueing should be made upon smaller surfaces of castings, and upon parts that are not to be subjected to strains or stresses. For inlays, instead of sprueing at the contact point or the marginal ridges—the usual practice—the sprue should be placed upon cavo-surfaces or surfaces that can be easily

cleaned. In larger castings the same principles should be kept in mind. The use of more than one sprue doubles the area of porosity. Additional sprueing, if necessary, should be placed with extreme care.

The second variation in hardness is one that has not been considered in dentistry. The alloys of all metals that form solid solutions, such as gold and copper, gold and silver, or gold and platinum, do not solidify to a uniform chemical composition. Tammann has pointed out that, for the ordinary rate of cooling, the crystallites of the solidified alloys are not homogeneous. In gold-copper alloys containing 0 to 82 percent of gold, by weight, the center of the crystallites is richer in copper than the peripheral layer; in alloys containing 82 to 100 percent of gold, this distribution is reversed. From the copper-rich melts, the solid solutions that separate first are richer in copper than the melt, the remainder of the melt becoming higher in gold. At the crystallization of the gold-rich melts, the relations are reversed, the center of the cored crystals consequently being richest in gold. *Figs. 4, 5* and *6* show this segregation. *Fig. 4* is from the region at the end of the casting. *Fig. 5* is the same casting, at the center, showing equal amounts of gold-rich and copper-rich solid solutions. *Fig. 6* is a view near the sprue, showing predominance of copper-rich solid solution. The portions of greatest hardness were those nearer in composition to the 50-50 percent atomic gold combination, at which transition region there is formed a compound of cupric-auride (CuAu), the hardest formation in the whole copper-gold series. These masses, being composed of more fusible portions of the alloy, rise into that part of the casting which is last to solidify. Therefore, into these hardness curves we read the second factor of variation—due to this segregation. When, however, the alloy is annealed at a temperature close to its melting point, the crystals become homogeneous and this hardness variable disappears. *Fig. 7*, illustrating the same alloy after it was annealed or homogenized at 875°C., shows complete diffusion of gold-rich and copper-rich solid solutions. The hardness curve again indicated only the variations due to the first variable described above.

General note. Alloy No. 9—15 percent gold, 85 percent copper—was selected for these illustrations not because of its practicability as a dental alloy but because of its suitability to show the important conditions of non-homogeneity that exist to a lesser extent in dental alloys. Furthermore,

this alloy, being low in gold and high in copper, is very easily oxidized during melting in the crucible as well as during the casting operation itself. Therefore, excessive oxidation, as evidenced by the occluded oxides and blowholes, is not typical of good dental practice.

The last variable to be discussed is that of precipitation-hardening. During the years 1903 to 1911, Wilm became aware of this phenomenon in his work on the hardening and strengthening of duralumin. In 1919, Merica, then in the Bureau of Standards, explained this precipitation hardness for specimens of duralumin from "Zeppelins" downed by the English during the war. In 1924, Jeffries and Archer presented the theory of the keying action of the compound particles that brought about this formation. Since then this phenomenon has played an important rôle in the metallurgy of many alloys. In the gold-copper system, this precipitation hardness is brought about by cupric auride (CuAu) as the precipitated compound. As stated above, this transition takes place in 50-50 percent atomic-gold alloys. At the optimum of this precipitation, this alloy demonstrates a hardness of 10.5 kilograms pressure for a depth of 1/200 mm., using a 2.5 mm. tungsten-carbide penetrator. Since under the same conditions the hardness for copper is 1.82 kg., and for gold is 1.02 kg., an increase of approximately tenfold over either of the alloying elements is shown. For the same alloy, when heated above the temperature of this precipitation-hardness formation (which is about 400°C. at a temperature of 600°C. for four hours and quenched in 25°C. water), the result was 6.2 kg. However quickly the alloy may be quenched, some of this transition always occurs.

For the ordinary rate of cooling, this precipitation hardness attains almost the same degree of hardness. Tests of the gold-copper system reveal that in alloys containing from 0 to 35 percent of atomic gold, and in those having from 70 to 100 percent of atomic gold, precipitation hardness is a very small factor. Given the same treatment that brings about maximum or minimum precipitation-hardness changes, the differences are less than 0.1 kg. Since this latter group includes the ratios used in dentistry, if no other element is used to bring about this formation this factor of precipitation hardness is negligible, and cannot be used for gold-copper dental alloys.

The authors' acknowledgement is due to Dr. W. H. Welker for his advice and criticism, and for his interest in our work. The authors are also indebted to Dr. V. T. Nylander for a loan, from the Infirmary, of a portion of the precious metals used in this work.

BIBLIOGRAPHY

(1) Allen, N. P. The distribution of porosity in copper ingots. *Institute of Metals Advance Copy*, 1933, No. 622.

(2) Bannister, C. O. Studies on the crystallization of gold from the liquid state. *Journal of the Institute of Metals*, 1929, *42*, 141.

(3) Capillon, Edward A. Melting and casting some gold alloys. *American Institute of Mining and Metallurgical Engineers; Technical Publication*, 1930, No. 282.

(4) Haughton, J. L., and Payne, Ronald J. M. Transformations in the gold-copper alloys. *Institute of Metals Advance Copy*, 1931, no. 571.

(5) Jeffries, Zay, and Archer, Robert S. The science of metals, First Edition; McGraw-Hill Book Company, Inc., N. Y.; 1924.

(6) Kurnakow, N. S., and Ageew, N. W. Physico-chemical study of the gold-copper solid solutions. *Institute of Metals Advance Copy*, 1931, No. 576.

(7) Kurnakow, N., Zemczuzny, S., and Zasedatelev, M. The transformations in alloys of gold with copper. *Journal of the Institute of Metals*, 1916, *15*, 305.

(8) Mercia, Paul D. The age-hardening of metals. *Transactions of the American Institute of Mining and Metallurgical Engineers*, 1932, *99*, 13.

(9) Rosenhain, Walter. Introduction to Physical Metallurgy, Second Edition; Richard R. Smith, Inc., N. Y., 1930.

(10) Tammann, Gustav. A text book of metallography. Translated from the third German edition, by Reginald Scott Dean and Leslie Gerald Swenson; Chemical Catalog Company, Inc., N. Y.: 1925.

(11) Wise, E. M., Crowell, W. S., and Eash, J. T. The rôle of the platinum metals in dental alloys. *Transaction of the American Institute of Mining and Metallurgical Engineers;* Institute of Metals Division, 1932, *99*, 363.

A QUANTITATIVE METHOD FOR ESTIMATING BACILLUS ACIDOPHILUS IN SALIVA[1]

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III. Quantitative determination of B. acidophilus in caries-susceptible and caries-free children 421
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I. INTRODUCTION

Recent work on the relationship of B. acidophilus to dental caries has developed the need for a special method for quantitative determination of B. acidophilus in the mouth. This has become especially important for further work along two lines; namely, use in comparative studies of the incidence of B. acidophilus in caries-susceptible and caries-free subjects, and in connection with attempts to reduce, by various prophylactic and dietary measures, the aciduric flora of the mouth in extreme cases of caries. Earlier work on these two aspects of dental caries has been carried out solely by qualitative means, the only available criterion for the presence of B. acidophilus in the mouth having been its growth in acid glucose-broth cultures. This test is referred to as qualitative in the sense that either a few organisms or several thousand, in the material inoculated, give the same final reading. This relatively crude method has, however, revealed striking differences between caries-susceptible and caries-free subjects. In cases of active caries, positive cultures of B. acidophilus

[1] This work was carried out under a grant for dental-caries research, from the Children's Fund of Michigan; Dr. R. W. Bunting, Director. Proceedings of the eleventh general meeting of the International Association for Dental Research: *Journal of Dental Research*, 1933, xiii, p. 198.

are almost invariably obtained from tooth-scrapings and from saliva, although a majority of subjects continuously free from caries show negative cultures, particularly from tooth-scrapings (1). Our findings have been confirmed by Thompson (9), and Enright, Friesell, and Trescher (2), but were not verified by Tucker (10). It has been our opinion that, in the small number of caries-free cases from which positive broth cultures have been obtained, determinations of the actual number of B. acidophilus in the inocula would have shown a striking contrast to the number present in the inocula from caries-susceptible cases. If this were true, it might explain why some caries-free individuals more or less constantly give positive cultures of B. acidophilus and yet do not develop caries; for, if only a few of these organisms are present in the mouth, the fact indicates that conditions there are not conducive to their growth, and implies that caries will not develop unless a sufficient number of organisms are present to produce the required amount of acid.

With regard to study of the effect of clinical treatment on the aciduric flora of cases of extreme caries, it is obvious that the qualitative broth-method does not reveal gradual numerical decrease of these organisms. A method of quantitative examination is essential for studies of this character.

II. METHODS

Rodriguez (7) recently devised a medium by which, he believed, colonies of B. acidophilus could be recognized and differentiated from those of other mouth organisms. The medium consists of 10 percent horse-serum agar (pH 7.2), which, after inoculation, is incubated anaerobically in an atmosphere containing 10 percent of carbon dioxide. It was stated that by this method B.-acidophilus colonies developed an opaque halo, which the organisms that produced less acid did not exhibit. In our laboratory, however, this test has not given dependable results, since colonies other than those of B. acidophilus frequently showed the halo. Many special media have been recommended for the cultivation of B. acidophilus, from the intestines or from milk. It was considered possible that one of these might give the differential results required for studies on oral acidophilus. The casein-digest agar of Kulp and Rettger, the vegetable-peptone agar of Bachman and Frost, and the tomato-peptone agar of Kulp, are the better known. Any of these may be used for the enumeration of B. acidophilus in commer-

cial-milk preparations, in which the organism is present in pure culture. We have found, however, that none of them permits a satisfactory differentiation of oral B. acidophilus from certain other mouth bacteria since several cultivable mouth species, particularly the streptococci, grew as well as acidophilus on these media.

Howitt and Fleming (4), in their excellent quantitative study of mouth flora in pyorrhea, made use of anaerobic glucose veal-infusion agar-plates (pH 4.8–5.0) for the estimation of gram-positive, aciduric rods. Our attempt to use a glucose beef-infusion agar (pH 5.0, to restrain the growth of non-acid-resisting mouth bacteria) resulted in the difficulty many have encountered (although not mentioned by Howitt and Fleming) when attempting to employ agar media at high alkaline or acid reactions. Under these conditions the jellifying characteristic of agar is destroyed, if heated at high temperatures. However, if the agar is acidified after sterilization and cooling (using aseptic precautions), and plates poured at once, the usual solidification takes place. This method is obviously time-consuming and impracticable for general use. We found that a medium containing 4 percent of agar remains solid after autoclaving, if lactic acid instead of hydrochloric is employed for adjustment. This medium proved unsatisfactory, however, because colonies of B. acidophilus remained too small to permit accurate counts.

A. *Culture medium employed.* We found that Kulp's (6) tomato-peptone agar could be adjusted to pH 5.0 without altering its consistency. The only changes made by us in Kulp's formula were (a) use of 2 percent agar instead of the recommended 1.1 percent, and (b) adjustment of the reaction to pH 5.0, instead of 6.2, by addition of lactic acid. This medium had the following advantages for our purposes: (*a*) The combination of filtered tomato juice, peptonized milk, peptone, agar, and water brings the initial reaction to about pH 5.4. Therefore, very little lactic acid (usually about 5 cc. of normal acid per liter of medium) is required to give a final reaction of pH 5.0. (*b*) Addition of 2 percent agar gives a medium that remains solid after autoclaving at 15-lb. pressure for 15 minutes. (*c*) It is very easily prepared, and gives a clear, yellow product. No addition of carbohydrate is required. (*d*) All the colony phases of B. acidophilus, including smooth, intermediate, and rough, grow well and can be easily recognized. Most oral streptococci are inhibited; occasionally an aciduric strain grows, but its colonies can be differentiated from those of B. acidophilus by their pin-point size. A yeast-like organism, frequently present in the mouth, can tolerate this acidity, but its colonies are easily distinguished by their greater size (*figs. 2* and *8*). Colonies of M. tetragenus and

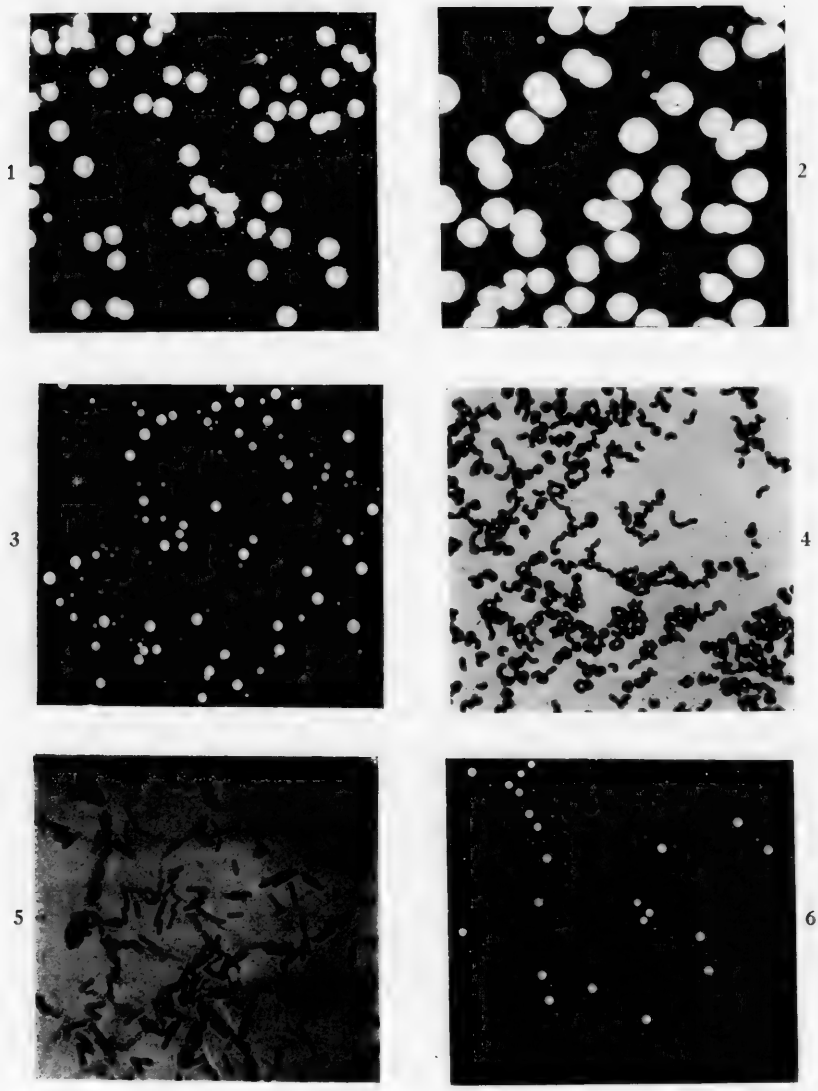


FIG. 1. Undiluted "caries-free" saliva (0.4 cc.) on glucose beef-infusion agar (pH 7.0). Large colonies, yeast; small ones, streptococci, staphylococci, and B. acidophilus. Impossible to count B. acidophilus colonies.

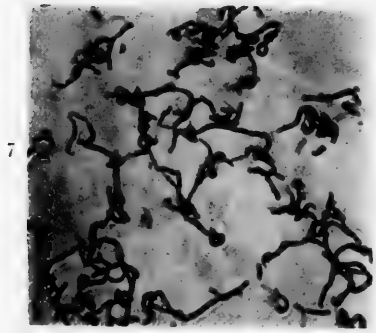
FIG. 2. Undiluted "caries-free" saliva (0.4 cc.)—same sample as in *fig. 1*—on tomato-peptone agar (pH 5.0). Large colonies, yeast. Small colonies, B. acidophilus—25 per cc. of saliva.

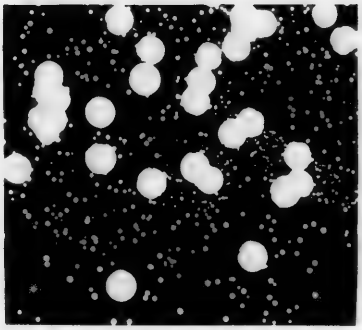
FIG. 3. "Caries-susceptible" saliva (0.1 cc. of 1–20 dilution) on tomato-peptone agar (pH 5.0). All colonies, B. acidophilus—23,000 per cc. of saliva. Smaller colonies, B. acidophilus, Type I; larger ones, B. acidophilus, Type II (note darker centers).

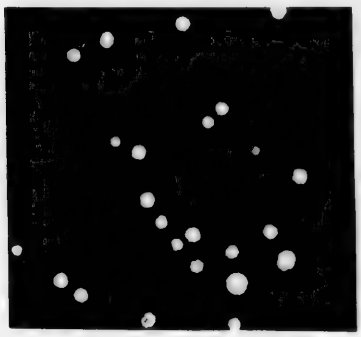
FIG. 4. B. acidophilus, Type I; organisms from Type-I colony in *fig. 3*.

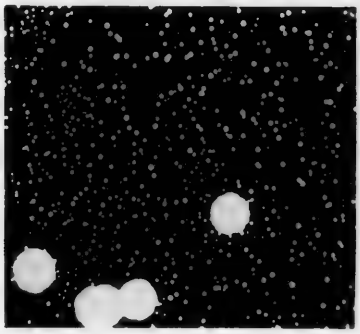
FIG. 5. B. acidophilus, Type II; organisms from Type-II colony in *fig. 3*.

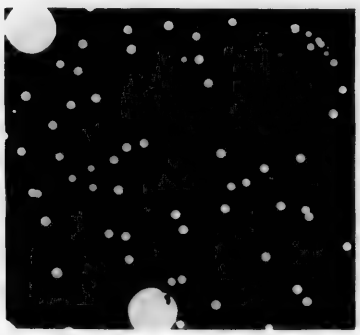
FIG. 6. "Caries-susceptible" saliva (0.1 cc. of 1–20 dilution) on tomato-peptone agar (pH 5.0). Larger, white colonies—B. acidophilus, Type I; smaller, gray ones—B. acidophilus, Type III.











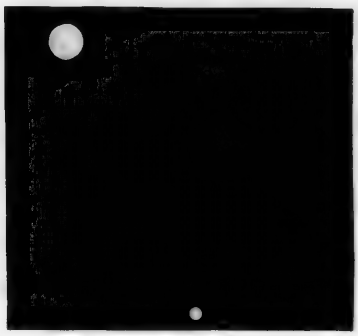


FIG. 7. B. acidophilus, Type III; organisms from Type-III colony in *fig. 6*.

FIG. 8. "Caries-susceptible" saliva (0.1 cc. of 1–10 dilution) on tomato-peptone agar (pH 5.0). Shows yeast and B. acidophilus colonies.

FIG. 9. Undiluted "caries-free" saliva (0.4 cc.) on tomato-peptone agar (pH 5.0). Shows M. tetragenus colonies only. Note irregular borders.

FIG. 10. Patient E. R. S.; caries-susceptible; high-carbohydrate diet. Saliva (0.1 cc. of 1–100 dilution) on tomato-peptone agar (pH 5.0); 1,800,000 B. acidophilus, and 4,000 yeast, colonies per cc. of saliva.

FIG. 11. Patient E. R. S.; low carbohydrate diet for 12 days. Saliva (0.1 cc. of 1–5 dilution) on tomato-peptone agar (pH 5.0); 4,500 B. acidophilus, and 400 yeast, colonies per cc. of saliva.

FIG. 12. Patient E. R. S.; low carbohydrate diet for 18 days. Saliva (0.1 cc. of 1–5 dilution) on tomato-peptone agar (pH 5.0); 100 B. acidophilus, and 50 yeast, colonies per cc. of saliva.

of Staphylococcus albus occasionally appear, and are similar in size to B. acidophilus, but with a little experience one soon finds it possible to differentiate them.

Figs. 1 and *2* show the result of spreading equal amounts of the same sample of saliva on two kinds of medium: glucose beef-infusion agar (pH 7.0) and tomato-peptone agar (pH 5.0). The inhibitory effect exercised on many mouth organisms by the latter medium is well illustrated. Only B. acidophilus and yeast colonies grew on the acid medium, whereas yeast, streptococci, staphylococci, and B. acidophilus were present on the neutral agar. *Figs. 3–7* show the three most common colonial and morphological types of B. acidophilus, from saliva, growing on tomato agar (pH 5.0). These were designated Types I, II, and III in an earlier publication (3). Types I and II form smooth colonies; Type III gives an intermediate type. The cell-forms associated with each colonial type are shown in *figs. 4, 5,* and *7*.

B. *Collection of sample.* For the purpose of enumerating B. acidophilus in mouths of caries-susceptible or caries-free patients, the following procedure is successful: The patient is instructed to chew a small piece of paraffin vigorously for three minutes, moving the paraffin alternately from side to side of the mouth to dislodge from the teeth as much deposit as possible. During this time the sample of saliva, collected in a tube graduated to 10 cc., usually varies in amount from 2 to 8 cc. Its volume is made up to 10 cc. with sterile physiological salt solution. The organisms dislodged by three minutes of chewing are therefore always suspended in the same amount of fluid, so that the error caused by the varying rate of flow of saliva is minimized. The tube is shaken by hand for 30 seconds, and is then ready for dilution.

C. *Dilution of sample.* To facilitate counting, it is necessary to adjust the dilutions of saliva to the relative numbers of organisms in the mouth of the caries-susceptible or the caries-free patient. For *caries-susceptible* cases: to each of two tubes, containing respectively 4 cc. and 19 cc. of sterile physiological salt solution, is added by pipette 1 cc. of saliva sample, thus giving dilutions of 1–5 and 1–20. After these dilutions have been thoroughly shaken, 0.1 cc. from each is placed on the center of a hardened and well-dried tomato-agar (pH 5.0) plate. With a sterile, bent glass-rod, the inoculum is spread evenly over the surface. Plates are incubated, inverted, at 37°C. for three days, and the colonies then counted. The final count from the 1–5 dilution must be multiplied by 50, and that from the 1–20 dilution by 200, to give the number of bacteria per cc. of saliva. After the first test has been made, it is often necessary in subsequent tests to alter

the dilution for a certain patient, if the colonies are found to be either too sparsely distributed or too crowded. It may thus be necessary to spread on the agar 0.2 cc. of the 1–5 dilution of saliva from a patient with a very small amount of caries, while 0.1 cc. of 1–100 or 1–500 dilution may be sufficient for an extremely active case. The same dilution is usually satisfactory for future tests, unless treatment is administered for the purpose of reducing the aciduric flora. Under such circumstances the dilution must be lowered.

In the case of *caries-free* patients, no saline dilution of saliva is as a rule necessary (the volume correction to 10 cc. is made as in susceptible cases). Until a favorable range is determined for a given case, two plates are spread as above with 0.1 cc. and 0.4 cc. of the undiluted but well-shaken saliva sample, the counts being multiplied in such cases by 10 and 2.5 respectively. In a majority of instances, the 0.4-cc. plate remains free from growth of B. acidophilus, and is frequently sterile; larger amounts of saliva cannot satisfactorily be spread over the surface of the agar. The 0.4 cc. plate must be allowed to dry well before being inverted and incubated.

Figs. 8 and *9* demonstrate the distinct and characteristic difference between salivas from caries-susceptible and from caries-free individuals. The caries-susceptible plate (0.1 cc. of 1–10 dilution) shows both yeast and acidophilus colonies; the caries-free plate (0.4 cc. undiluted) shows only colonies of M. tetragenus.

III. QUANTITATIVE DETERMINATION OF B. ACIDOPHILUS IN CARIES-SUSCEPTIBLE AND CARIES-FREE CHILDREN

Using the quantitative method described above, a group of thirty-one children, 9–16 years old, was studied over a period of 18 months. Very careful dental examinations were made at the beginning of this period; also at the end of 12 months, and again at the end of 18 months.[2] Fourteen children during this time developed active caries (thirteen had previously had caries, while one had been free from caries up to this 18-month period). Ten children, who had always been free from caries, had no new lesions at the end of the 18 months. The remaining seven children became "clinically questionable;" that is to say, it was impossible to determine whether new caries had occurred during this time. For this reason the bacteriological findings on the last

[2] The dental examinations were made by Drs. R. W. Bunting, Dorothy G. Hard, and Philip Jay, of the School of Dentistry.

TABLE 1

*Incidence of B. acidophilus in caries-susceptible children during 18 months**

CASE	ACID BROTH		ACID AGAR	AVERAGE
	Tooth-scraping	Saliva	No. per cc. of saliva	
F. B.	+	+	56,100	
	+	+	68,100	
			2,800	
		+	14,000	
		+	9,050	
	+	+	28,400	29,742
L. F.	+	+	127,000	
	+	+	448,000	
			11,400	
		+	9,150	
		+	32,000	
	+	+	136,400	109,136
N. I.	+	+	48,500	
	+	+	138,000	
		+	2,600	
			2,750	
		+	4,575	
	+	+	116,400	52,137
R. L.	+	+	180,000	
	+	+	7,800	
			111,200	
		+	246,200	
		+	28,400	
	+	+	36,600	101,700
A. E.	+	+	5,000	
		+	4,900	
		+	100	
		+	5,900	
	+	+	42,700	11,720
J. P.	+	+	6,000	
	+	+	20,000	
			9,200	
			3,500	
		+	164,200	
	+	+	28,600	38,583
A. W.	+	+	151,150	
		+	303,600	
	+	+	276,000	243,583
G. R.	+	+	120,000	
	+	+	7,650	
			2,500	
		+	950	
		+	41,150	
	+	+	840	28,848
J. R.	+	+	16,000	
	+	+	24,000	
		+	700	
		+	1,620	
	+	+	1,180	8,700
W. W.	+	+	50,000	
	+	+	32,500	
		+	267,000	
		+	58,600	
		+	63,800	
	+	+	403,000	145,817
M. W.	+	+	7,260	
	+	+	23,800	
			33,200	
		+	15,050	
		+	10,250	
	+	+	28,000	19,593
J. Roy	+	+	24,000	
	+	+	3,300	
		+	41,600	
			10,000	
	+	+	36,400	23,060
P. S.	+	+	8,550	
	+	+	100,000	
			14,200	
			5,450	
			9,150	
		+	34,100	
	+	+	282,000	64,778
O. L.	+	+	3,200	
	+	+	48,800	
		+	900	
		+	3,150	
		+	16,200	
	+	+	15,800	14,675

* *Qualitative* tests are indicated by the acid-broth cultivations from tooth-scrapings and saliva. *Quantitative* tests are indicated by the number of colonies on acid agar.

TABLE 2

*Incidence of B. acidophilus in caries-free children during 18 months**

CASE	ACID BROTH		ACID AGAR	AVERAGE	CASE	ACID BROTH		ACID AGAR	AVERAGE
	Tooth-scraping	Saliva	No. per cc. of saliva			Tooth-scraping	Saliva	No. per cc. of saliva	
P. B.	−	−	0		M. R.	−	−	0	
	−	−	0			+	+	160	
	−	−	0					2	
		−	0				−	0	
		−	0				−	0	
		−	0				+	0	
	−	−	0	0		+	+	12	25
R. P.	+	+	0		J. E.	−	+	210	
	−	−	0			−	+	50	
	−	+	0			−	+	585	
	−	−	0				+	45	
		−	0				+	117	
		−	0				+	27	
		−	0				+	1020	
		+	0			+	+	0	256
	−	+	20	2					
Jo. E.	−	+	0		V. D.	+	+	0	
	−	+	0			−	−	0	
		−	0				+	45	
		−	2			−	−	0	
		−	0				−	0	
	−	+	7	1		+	+	385	72
W. G.	−	−	0		M. B.	−	−	0	
	−	−	0			−	−	0	
	−	+	30				+	3700	
		−	0				+	905	
	−	+	5				+	70	
		+	0			−	−	0	779
	−	−	5	6					
M. S.	−	−	5		E. B.	+	+	800	
	−	+	15		(Negro)	−	+	7600	
			2				+	19,200	
		−	0			+	+	120	
		−	0				+	1680	
		−	0			−	+	0	4900
	−	−	0	3					

* *Qualitative* tests are indicated by the acid-broth cultivations from tooth-scrapings and saliva. *Quantitative* tests are indicated by the number of colonies on acid agar.

group are omitted from the present report.[3] Cultures were made from the saliva of each child at irregular intervals over the 18-month period. Six months elapsed between some cultivations; only one month, between others. At least six cultures were taken from a majority of cases: the lowest number from any one case was three; the highest, nine. In addition to quantitative acidophilus-counts from saliva, qualitative cultures were made, in the usual 1 percent glucose beef-infusion broth (pH 5.0), from tooth-scrapings and from saliva. For the latter, 0.4 cc., from both caries-susceptible and caries-free cases, was inoculated into broth.

The striking difference, in acidophilus counts, between caries-susceptible and caries-free cases is shown in *tables 1* and *2*. There were, in all, 79 separate quantitative examinations in the susceptible group; 69 in the caries-free group. In the susceptible group, 100 percent of these examinations were positive for B. acidophilus; in the caries-free group, only 42 percent were positive to any degree. The average count for the 14 caries-susceptibles (total of 79 examinations) was approximately 60,000 B. acidophilus per cc. of saliva, whereas that for the 10 caries-free cases (total of 69 examinations) was 600. The range of individual counts for the susceptible group was extremely wide, varying from a few hundred to 500,000, *with a majority over 20,000*. In the caries-free group the range was from 0 to 20,000, *a majority of counts being 0—in 81 percent the counts were less than 100*. It will be noted that the counts for each individual varied considerably, particularly for those in the susceptible group. This may be due to the fact that several months sometimes elapsed between examinations. Besides, it is practically impossible to obtain uniform counts from one individual even from one day to the next, since the paraffin chewing cannot regularly dislodge the same number of organisms from the teeth. Until a method for obtaining more uniform samples is worked out, this method is useful chiefly for comparative purposes.

While only one caries-free case stands out as being completely acidophilus-free during the 18 months, six of the remaining cases show counts so low as to be practically negligible, their average being under 75. The remaining three have somewhat higher averages (256, 779

[3] This same group of children was discussed in a recent paper by Hubbell (5) on chemical studies of saliva in relation to dental caries.

and 4,900, respectively), although in all three cases low or negative counts were frequently obtained. *Case E. B.*, showing the highest figures for the caries-free group, was the only Negro. All the caries-free cases showed one or more negative counts during the period of observation, thus indicating the transient nature of the infection in these cases.

Comparison of the broth cultures from tooth-scrapings and from saliva (our usual method of determining the presence of B. acidophilus in the mouth) shows that all in the susceptible group were positive. In the caries-free cases, however, there were many more positive cultures from saliva than from tooth-scrapings. Of the 38 instances in which broth-cultures were made from tooth-scrapings and from saliva, only 8 cultures from scrapings were positive, while 21 from saliva were positive. An illustration of this appears in *Case J. E.* (*table 2*), in which the first three tooth-scraping cultures were negative, whereas all the saliva broth-cultures, made at the same time, were positive. The saliva counts, however, were very low—210, 50 and 585. It therefore appears that positive broth-cultures from caries-free salivas do not necessarily indicate high acidophilus overgrowths, since frequently the actual number of organisms is low, and tooth-scrapings may be negative. Apparently in such cases the organisms are not localized, or are localized only to a small extent on the teeth, but are free in the saliva. It also should be noted that, in the caries-free cases, broth cultures from saliva were sometimes positive for B. acidophilus although agar plates inoculated with the same quantity of saliva were negative. This may be explained by the fact that acid glucose-broth serves as an enrichment fluid for B. acidophilus, a very small number of these organisms being stimulated to growth in broth, whereas on the surface of an agar plate there is greater initial mortality.

IV. REDUCTION OF B. ACIDOPHILUS BY REGULATION OF CARBOHYDRATE IN DIET

The quantitative method for estimating B. acidophilus in saliva may also be employed advantageously in the evaluation of attempts to control caries by limiting the growth of this organism in the mouth. Howitt and Fleming (4) reported that a diet predominantly carbohy-

drate increased the number of aciduric organisms in the mouth, whereas a diet containing more protein diminished their number. Rodriguez (8) was also able to decrease the number of these organisms by reduction of carbohydrate in the diet. This method was used by us successfully on several cases, one of which will be described.

The patient, age 24, was extremely susceptible to dental caries, several new cavities developing each year in spite of continued dental attention. Before any change in diet was undertaken, quantitative estimations of B. acidophilus were made several times a week for six weeks. During this time the counts varied from a low of 40,000 per cc. of saliva to a high of 300,000, with a usual count of approximately 150,000. The patient was then advised to use a very *low* carbohydrate diet, which was prescribed by Dr. Martha Koehne of our research group. All sweet desserts, candy and sugar were eliminated, as well as starchy foods such as bread, potato, and certain vegetables. After seven days on this diet, the count was reduced to 10,000 B. acidophilus per cc. of saliva; in 4 weeks the count dropped to 900. The patient then returned with much enthusiasm to a carbohydrate diet, again consuming the usual amounts of sugar and starch, whereupon the acidophilus count rose in five days to 1,800,000 per cc. of saliva (*fig. 10*). The count remained above 1,000,000 for several days, during which time large amounts of carbohydrate were consumed. Next, a *moderate* carbohydrate diet was begun (limited quantities of bread and potato allowed, but sugar and sweet desserts eliminated). Since ten days on this diet reduced the count only to 130,000, the very *low* carbohydrate diet was again prescribed, whereupon the count dropped to 4,500 in twelve days (*fig. 11*), and to the extremely low figure of 100 in eighteen days (*fig. 12*). When more carbohydrate was added to the diet, the count again rose and remained around the 150,000 level.

This case illustrates how the quantitative method of estimating B. acidophilus in saliva may be applied to procedures designed to control dental caries. It shows, moreover, what may be accomplished in reducing acidophilus flora by rigid control of carbohydrate in the diet. It was not possible to determine the effect of this carbohydrate reduction on the progress of the disease in this particular case, since long continued adherence to the limited, and rather costly, diet was not advisable. Details regarding other cases, in which carbohydrate in-

take was controlled over longer periods of time, will be reported by Dr. Martha Koehne.

V. SUMMARY

The need for a solid medium for quantitative determinations of oral B. acidophilus in connection with studies on the bacteriology of dental caries has been met by devising a modification of Kulp's tomato-peptone agar.

This modification involved increasing the acidity of the medium to pH 5.0, with lactic acid, and the agar content to 2 percent.

On this medium colonies of B. acidophilus of the smooth, rough, and intermediate phases may be differentiated from each other, and from the other mouth organisms, such as yeast, staphylococcus, M. tetragenus, and occasional streptococci, which tolerate this acidity. Most streptococci and staphylococci do not grow.

Methods for collection of saliva, dilution of the sample, and spreading on the plates are described. The number of B. acidophilus per cc. of saliva can thus be estimated.

In order to secure well-distributed colonies on agar plates, saliva from caries-susceptible patients requires a much higher dilution than does saliva from caries-free patients. In the latter group it is often necessary to employ undiluted saliva.

A group of fourteen caries-susceptible and ten caries-free children was cultured at intervals for 18 months. In the *susceptible* group, qualitative cultures showed B. acidophilus present in all cases from tooth-scrapings and from saliva. Quantitative estimations ranged from a few hundred to 500,000 per cc. of saliva, with a majority over 20,000. The average for 79 examinations was 60,000.

In the *caries-free* group, qualitative cultures showed B. acidophilus present in only 21 percent of tooth-scrapings, but in 51 percent of salivas. Quantitative estimations of B. acidophilus in saliva ranged from 0 to 20,000, with a majority of counts 0—in 81 percent the counts were less than 100. The average for 69 examinations was 600.

While B. acidophilus is invariably present, and usually in great numbers, in the mouths of caries-susceptible subjects, in caries-free individuals its presence is variable and usually marked by few organisms.

By reducing carbohydrate in the diet to the lowest possible minimum, in a patient extremely susceptible to caries, B. acidophilus was decreased in 18 days from the very high figure of 1,800,000 per cc. of saliva to 100 per cc.

The advantage to be gained from applying this quantitative method to comparative studies of the aciduric flora of caries-susceptible and caries-free mouths, and to studies of methods of control of aciduric overgrowths, is indicated.

VI. REFERENCES

(1) Bunting, R. W., and Palmerlee, Faith. 1925 *Jour. Am. Dent. Ass'n.*, *12*, 381.
(2) Enright, J. J., Friesell, H. E., and Trescher, M. O. 1932 *Jour. Dent. Res.*, *12*, 759.
(3) Hadley, Faith P., Bunting, R. W., and Delves, Edna A. 1930 *Jour. Am. Dent. Ass'n.*, *17*, 2041.
(4) Howitt, Beatrice F., and Fleming, W. C. 1930 *Jour. Dent. Res.*, *10*, 33.
(5) Hubbell, Rebecca B. 1933 *Am. Jour. Physiol.*, *105*, 436.
(6) Kulp, Walter L., and White, Vinton 1932 *Science*, *76*, July 1, 17.
(7) Rodriguez, F. E. 1930 *Jour. Am. Dent. Ass'n.*, *17*, 1711.
(8) ——— 1931 *Ibid.*, *18*, 2118.
(9) Thompson, Richard 1931 *Proc. Soc. Exp. Biol. and Med.*, *29*, 103.
(10) Tucker, Winston H. 1932 *Jour. Inf. Dis.*, *51*, 444.